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THE LIQUID CHROMATOGRAPHY OF LIPIDS

A CRITICAL REVIEW

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CONTENTS

1. INTRODUCTION

The chromatography of lipids has been described and reviewed[1–6] several times and therefore a further review such as this requires some definition of its scope.

Liquid chromatography (LC), in its modern form of high-speed or high-performance liquid chromatography (HPLC)[7–11], has advanced very rapidly during the past few years. Much of this upsurge is due to the increasing availability of commercial LC equipment for high-pressure chromatography in narrow-bore analytical columns[12–16]. However, many researchers in the field of lipids, fats and oleochemicals felt that the commercially available equipment did not fully satisfy their requirements and they therefore either tried to build their own equipment, to modify existing pieces of equipment, or to use only certain parts of a liquid chromatograph. This survey describes what has been attempted and what has been achieved in this respect.

In the literature describing applications there are a number of papers where the authors used only partial HPLC equipment. Some workers did not have access to a suitable detector but used high-pressure equipment. Other workers had a detector that was capable of monitoring a lipid eluate continuously, but did not use a pump for a variety of reasons, *e.g.*, because their separation was dependent on a compressible column filling material. A number of these papers are included in this review, *e.g.*, part of the low-pressure work using instrumentation that is more advanced than the conventional gravity-flow column plus fraction collector.

As the separation techniques as such and considerations regarding detectors and other LC equipment are often very similar, this review is not limited to the LC of biological lipids but also includes food lipids and some technical fats and oleochemicals.

In some areas of marginal interest, *e.g.*, in the HPLC analysis of pharmaceutical preparations (tablets) of lipid-soluble vitamins and steroids, this review is intentionally not comprehensive. However, most of the key papers have been quoted.

Most of the references have been arranged near the end of the paper in the form of a table (Table 1), which illustrates the techniques that have been used for different applications.

2. EQUIPMENT

A. General

For information on the basic components of a modern liquid chromatograph, and for technical details, *e.g.*, on pumping systems and detector construction, performances, sensitivities, etc., the reader is referred to the basic LC literature[7–11], technical reviews[12–16] and to the appropriate company publications.

It seems to me that the LC of lipids differs considerably from the remainder of liquid chromatography on a number of counts that deserve comment and explanation. Most commercial HPLC equipment seems to have been designed with pharmaceutical, synthetic organic and polymer applications in mind. The major

differences concern the detector and gradient elution systems. If one compares publications on the LC of lipids with typical publications on "normal" LC, one may note the following points concerning the LC of lipids:

(a) Gradients or stepwise elution by more than two solvents are frequently used to cover a wide polarity range (in normal LC only two solvents are used).

(b) Special solvent effects[6] (and sorbent effects) seem to be exploited more frequently; this again often calls for more, and different, solvents used in stepwise succession (in normal LC only two solvents are used, involving a "polarity" gradient and "eluotropic series" concept).

(c) Gradients are usually formed on the low-pressure side of the pump, whereas in normal LC they are formed on the high-pressure side.

(d) Group or class separations predominate[1-3,5,6], whereas in normal LC individual substance separation and detection predominates.

(e) UV and RI detectors, as found in most commercial LC equipment, are of little use for the detection of lipids under gradient elution. The transport flame ionization detector is frequently favoured, whereas in normal LC, UV and RI detectors are favoured.

(f) Non-selective detectors of fairly *equal* sensitivity for most of the substances contained in a class of lipids are often preferred over selective detectors that show high sensitivity for only a few substances. In normal LC, emphasis is placed on high sensitivity at the expense of universality.

(g) Adsorption seems to be used more frequently[1], whereas in normal LC, partition and reversed-phase partition are more popular.

(h) Larger sample sizes seem to be used more frequently.

B. Detectors

The search for a lipid-sensitive detector is perhaps the most noteworthy aspect that becomes apparent on studying the literature. Most commercial LC apparatus is equipped with UV and RI detectors[12-16], but neither of these types is suitable for the detection of non-UV-absorbing samples under conditions of gradient elution. It seems that a solution to this problem can be found only when the solvents are removed (*e.g.*, evaporated) prior to detection of the solute. While this problem is particularly relevant in the LC of lipids, it is not related to lipids alone but to all non-UV-absorbing samples for which gradient elution may be required (*cf.*, carbohydrates and polysaccharides, *e.g.*, food additives, thickening agents and gums).

(a) Transport ionization detector systems

Transport flame ionization detectors (FIDs) are usually based on the principle of deposition of a portion of the eluate on a moving chain, wire, tape, band or disc, followed by evaporation of the solvent. The deposit of the solute is then transported into the flame of an FID, either directly or after vaporisation, pyrolysis or other transformations such as oxidation to carbon dioxide, followed by catalytic reduction to methane. In the latter instances the vapour, pyrolysis products or methane are swept into the FID by a stream of inert gas or hydrogen.

The first transport FID systems (and transport argon ionization detectors)

were described by Lieberman[17], Haahti and co-workers[18,19], James *et al.*[20,21], Karmen and co-workers[22–24], Stouffer and co-workers[25–27] and Anderson and Stevens[28]. Since then, similar detectors based on the transport FID principle have been described and tested by Maggs and Young[29–33], Scott and Lawrence[34], Pye[35], Cotgreave[36], Johnson *et al.*[37], Coll *et al.*[38], Dubský and co-workers[39–41], Stolyhwo *et al.*[42], Owens *et al.*[43], Lapidus and Karmen[44], Karmen *et al.*[45], Balaukhin *et al.*[46], Stevens[47,48], Savinov *et al.*[49], Foster and Weiss[50], Szakasits and Robinson[51] and Burnev *et al.*[52].

Other workers[53–55] have tried to improve the sensitivity of moving-wire detectors, and considerable improvements may be expected in the near future.

In the past, a number of these transport FIDs became commercially available[16], such as the following models: Pye Unicam (System 1, System 2); Pye Unicam (LCM-2); JEOL (JLC-FK); Packard (Model 7101); Nester-Faust (NFLC-400); and Barber-Colman (Models 5400, 5401; later Nuclear-Chicago Model 5402). A transport electron capture detector (ECD, System 3) was at one time also offered by Pye Unicam. For details of these detectors, the reader should consult the appropriate company literature. It seems that only a few of these commercial models were sold in larger numbers, *viz.*, the Pye Unicam moving-wire detectors[34], and most of the others now seem to be unavailable. All of these types of detector, however, are capable of monitoring lipid-containing eluates from gradient elution chromatography columns. Privett *et al.*[56] discussed the merits of several types of transport FID.

The Pye Unicam LCM-2 methane conversion moving-wire detector has advantages for the quantitation of peaks representing lipid classes, but seems to contribute to peak broadening so that part of the column efficiency is lost in the detector.

Most transport detectors today still seem to suffer from mechanical problems during prolonged use, and their reliability, reproducibility and quantitative performance, particularly during gradient elution, is not yet well established. Their sensitivity usually is lower than that of the UV detector, but some advanced models[34] compare favourably with the sensitivity of RI detectors, or even approach that of UV detectors[54]. A limitation is that a large difference in volatilities between the solvent and the solute is required. However, this is usually the case when LC comes into consideration, because gas–liquid chromatography (GLC) should be preferred for all more volatile samples, except when they are thermally labile.

Transport detectors are most popular whenever lipid extracts covering a wide range of polarities have to be analyzed by gradient elution (Table I).

(b) RI detectors

Differential refractometers have frequently been used for the detection of lipids in eluates from liquid chromatographic columns (Table I). RI detectors are supplied with most integrated commercial liquid chromatographs and are applicable whenever isocratic conditions are used. As this is always the case in true gel filtration or gel permeation chromatography (GPC), the RI detector is the detector of choice for all GPC separations of lipids. However, see the precautions mentioned in Section 5 on Quantitation.

In addition to GPC, the RI detector is also often used for the LC of lipids that are closely related, so that gradient elution is unnecessary even in adsorption[57,58] and argentation chromatography[59–62].

Sometimes the use of gradients formed from a "weak" solvent and a "strong" solvent, which both have approximately the same refractive index, is advocated[63,64]. In this instance, a differential RI detector can be used even during gradient elution, although only with reduced sensitivity. However, the method, which may be applicable to the gradient elution LC of lipids, has not found widespread use up to now.

(c) UV detectors

UV monitors are used mostly for the detection and quantitation of individual substances. UV-absorbing compounds are found mostly in the unsaponifiable portion of lipid extracts[65–67] and in the oxidation products of unsaturated lipids[68–70].

Both fixed-wavelength and variable-wavelength UV detectors are now available and have been used very frequently for the LC analysis of lipid-soluble vitamins (Table I). They are also useful for the detection of compounds with conjugated C=C and C=O groups that are formed during the oxidation of lipids[69,70] and in used frying fats[68,69].

Similar functional groups in some steroids and prostaglandins of mainly medical and pharmaceutical interest make these substances detectable by measuring their UV absorption (Table I). The same is true for carotenoids[71,72] and for proteins, which may occasionally be contained in a lipid extract. In lipoprotein mixtures, only the protein moieties will be detected by the UV detector.

Most of the antioxidants that are sometimes added in order to stabilize unsaturated edible fats are detectable by measuring their UV absorption[65,73–75].

As UV detectors are frequently more sensitive than other types of LC detector a number of attempts have been made to convert UV-transparent samples into UV-absorbing derivatives. Typical derivatives are benzyl[76], *p*-nitrobenzyl and 2-naphthacyl esters[77] for fatty acids, benzyloxime derivatives[78] and 2,4-dinitrophenylhydrazones[78–81] for carbonyl-containing steroids, and benzoate and *p*-nitrobenzoate esters for hydroxy compounds[80,82], including ceramides and other glycolipids[83–85]. For compounds with allylic hydroxyl groups, oxidation to the conjugated ketone has been used[86]. Derivative formation can be carried out either before or after the separation. In the former instance, a separation technique must be found for the derivatives, and a major advantage of LC over GLC is lost, *viz.*, the direct separation capability of underivatized (polar, non-volatile) samples. In the latter instance, derivatives can be formed by continuous[6,87] or discontinuous automatic[88,89] addition of a reagent after the elution of the separated substances from the column. In this way, however, part of the separation efficiency is lost owing to re-mixing of adjacent peaks in the large dead volumes.

(d) Detectors based on other principles

A large number of detectors based on principles other than those mentioned above have been described and tested for their use in liquid chromatography. They include radiochemical, polarographic, coulometric, heat of adsorption, electrical conductivity, thermal conductivity, electrical capacitance, differential viscosimetry, fluorescence, "heat of wetting", electron capture, ultrasound, IR and vapour pressure detectors, and they may all be applicable to the detection of lipids under certain circumstances. Examples are radioactivity detectors[71] for labelled lipids, polarographic detectors for vitamins such as the tocopherols or lipid quinones, and fluorescence

detectors for vitamin A[87] and E[90,91]. An IR laser has been used for the detection of serum lipids in high-speed LC[92].

A few of the newer developments are commented on here, either because they show high sensitivity for lipids or promise of future improvements, or because they are applicable to lipids under gradient elution.

A device that may be called a "transport RI" detector has been described by Werthessen *et al.*[93]. After evaporation of the chromatographic solvents, the sample is re-dissolved in another solvent and the refractive index of the newly formed solution is measured. The concept is attractive not only because gradient elution can be used, but also because the second solvent can be chosen, independent of its chromatographic properties, so as to give the maximum difference in refractive index between solvent and sample, and thus a better detection limit.

A detector in which a moving carrier plus gas–liquid partition was used was described by Haahti and Spaans[94].

A universal detector based on a piezoelectric quartz mass sensor was described by Schulz and King[95]. As the chromatographic solvents are evaporated, the device should also be applicable to lipids under gradient elution. The same is true for detectors that involve direct weighing of the (evaporated) eluate[96,97].

The "evaporative analyzer" principle described by Ford and Kennard[98] should also be applicable to lipids under gradient elution because the mist that gives the light-scattering effect is obtained upon evaporation of the solvent.

A transport mass spectrometric (LC–MS) detector of the moving-wire–electron impact ionization quadrupole MS type[99] should be very well suited to use with gradient elution. Scott *et al.*[99], for example, carried out LC–MS analyses of mixtures containing triacontane, cholesteryl laurate and cholesterol, of a fermentation extract and of a vitamin A mother liquor. Other LC–MS combinations in which chemical ionization (CI) is used are under development by several groups but may not be able to handle gradient eluates because the CI conditions, and the spectra obtained, may change with the solvents used.

Infrared absorption detectors monitoring the ester C=O band (and free OH bands, where applicable) have been used for the detection of lipids in GPC[100].

The spray impact detector described by Mowery and Juvet[101,102] has a high sensitivity for free fatty acids and fatty acid-derived surfactants. This detector, and the flame aerosol detector[102], which is claimed to be a universal detector with detection limits similar to those of UV detectors, are applicable only to reversed-phase HPLC.

A transport optical (light-scattering) detector for lipids under gradient elution was once considered by James *et al.*[103]. A diagram of this device is reproduced in the review of Sjövall *et al.*[104].

A simple evaporation ECD has been demonstrated to be useful for gradient elution[105]. Transport argon ionization detectors and transport ECDs have been mentioned in the earlier section on *Transport ionization detectors*.

C. Columns and injection systems

The columns and injection systems used for the LC of lipids are those generally used in LC. However, the following general remarks can be made.

For the LC of lipids, mostly low to moderate pressures have been used. Injection heads involving septa are therefore very popular and the sample is usually injected directly into the column filling.

In HPLC, there is now a trend away from the 2-mm I.D. column towards larger diameters. The author has used 4-mm I.D. columns for the past 5 years, and 4 mm is now increasingly becoming the standard column I.D. for a number of LC instrument manufacturers. Columns of 6 and 8 mm I.D. are also used. There is also a trend away from pellicular column filling materials and towards completely porous sorbents of higher capacity.

Both of these trends appear to be favourable for the LC of lipids, because larger columns of higher capacity permit the injection of larger samples. Present-day transport detectors are often not so sensitive as UV or RI detectors and give the best results with larger samples. With lipids, we usually inject samples in the milligram range, or even up to 10 mg for the more simple group separations[106,107]. Although the decisive parameter is the final sample dilution (sample concentration) that is reached at the effluent end of the column, it seems that completely porous 4-mm I.D. columns have made it easier to work with lipids and a moving-wire detector.

D. Pumps

The choice of a pump for the LC of lipids will be influenced not only by the pressure requirements, but perhaps more often by the type of gradient elution envisaged for separations of lipid classes.

Many workers use reciprocating pumps of the piston or diaphragm type. These are a necessity if gradients are formed on the low-pressure side. Depending on the type of gradient-forming device used, the hub (dead volume) and/or the pump stroke frequency may be of special importance.

From the author's point of view, the ideal pump is one that has constant *suction* in addition to constant delivery, and that is capable of delivering constant solvent volumes regardless of the column back-pressure, which usually changes* during a gradient run. Such a pump does not exist, but a pump of the high-frequency plunger type may be a fair approximation.

For isocratic operation, the ideal pump is probably a large-piston, forced-displacement pump that will deliver a constant flow at high pressures.

Part of the work reviewed here was carried out without the aid of a pump, particularly the work with compressible gels such as Sephadex derivatives. However, gas-pressurized solvents are frequently used (Table I).

Peristaltic pumps are usually not resistant to solvents, but several workers[108–111] have used such pumps for partition LC on Sephadex derivatives.

E. Gradient elution techniques

Gradient elution is especially important in the LC of lipids[1,5]. Lipid extracts, *e.g.*, of the chloroform–methanol type, may contain substances with a wide range of polarities, which may include hydrocarbons, carboxylic acids, glycolipids containing

* During a polarity gradient (*e.g.*, from a hydrocarbon to 30% ethanol) on silica gel, the column back-pressure may increase considerably, *e.g.*, by a factor of 2 or more.

a large number of free hydroxyl groups, and ionized molecules containing free phosphoric acid residues or quaternary ammonium ions. This situation is different from most of the non-lipid, "normal" LC applications.

In the analysis of lipids, much information has been accumulated over the years in conventional column and thin-layer chromatography (TLC) utilizing adsorption on silica gel[2]. Because of this background experience, there is a tendency to continue to use adsorption chromatography plus stepwise or gradient elution, even though liquid–liquid partition, and particularly reversed-phase partition, may frequently also be applicable.

The wide range of polarities is only one of the reasons why many researchers studying lipids feel that gradient elution with only two solvents, as suggested by most LC instruments manufacturers[12–15], is not sufficient. More than two solvents are frequently used to cover the range from pentane to methanol plus 8% aqueous ammonia[112]. When too few solvents are used, there may be "displacement effects"[113], or even problems of immiscibility.

The other reason is solvent selectivity, a subject that has been excellently reviewed by Rouser[6]. For example, proton-acceptor-only solvents such as acetone or ethyl acetate can be used to elute selectively glycolipids with several hydroxyl groups from a silica gel column without elution of phospholipids.

For separations of phospholipids from an anion-exchange column, the following sequence of gradients may be used[6]:

Gradient 1: chloroform to methanol in chloroform;

Gradient 2: methanol–chloroform to acetic acid in methanol–chloroform;

Gradient 3: methanol–chloroform–acetic acid to ammonium or potassium acetate.

The so-called Huber system*, although it may be able to cover a wide portion of the range of polarities found in the sample[114], may still not be able to satisfy the requirements for solvent selectivity. For example, it cannot provide for a changeover to a proton-acceptor-only solvent.

For gradient elution using only two solvents, where the gradient is formed on the high-pressure side of the pump(s), the reader is referred to the basic HPLC literature[7–15]. However, with lipids, gradient formation on the low-pressure side of the pump is frequently favoured[68,69,112,113,115–121], the major reason, apart from the fact that only one pump is needed, being that it is technically easier to pump several successive solvents or gradients through the column when the solvent switching or gradient formation is carried out on the low-pressure side.

Liquid chromatographic equipment for automatically producing steps or gradients of more than two solvents has been tested by Brusca and Gawienowski[122], Scott and Kucera[115], Aitzetmüller[68,118] and Stolyhwo and Privett[112], among others.

A useful system is the LKB Ultrograd[15] which is capable of producing automatically two gradients in succession (*e.g.*, solvents I → II followed by II → III) plus periods of column rinsing. In studies on lipids, it has been used for sequences of linear gradients[120] and concave gradients[68,76,77,118]. However, at present it is limited to three solvents.

* A ternary system[114] (coexisting phases of 2,2,4-trimethylpentane, ethanol and water) that is often recommended for gradient elution in liquid–liquid partition HPLC.

3. BRIEF DISCUSSION OF SEPARATION METHODS

A. The "modes" of liquid chromatography

Generally, there are four "modes" distinguished in LC, *viz.*, adsorption; liquid–liquid partition and reversed-phase partition (LLC); gel permeation; and ion exchange[7–11]. Frequently, however, two or more of these modes are operating at the same time and additive effects may be used in order to achieve the best separation factors.

Adsorption on silica gel is the traditionally favoured "mode" for the LC of lipids[1], but partition and GPC have also been widely used (Table 1). An important variation is adsorption on silver nitrate-impregnated silica gel columns[18,20].

Gel permeation is, by its very nature, mostly a "group separation" method (molecules of similar size are eluted together) and some of the precautions discussed below are applicable when quantitation is considered.

Partition and reversed-phase partition are often the methods of choice for separation within a lipid class (*i.e.*, a preparative class separation usually precedes it during the sample work-up procedure), and are most frequently used for steroids (Table 1). The Huber partition systems[114] are particularly useful for steroids.

True ion exchange is applicable only to acidic and basic or amphiphilic lipids. The use of ion-exchange columns is widespread in the HPLC of pharmaceuticals, but there have been very few reports of their application to the HPLC of polar lipids[123–125]. The reason is probably again the detection problem. There are, however, a number of studies in which ion-exchange columns have been used for partition[125] and adsorption charge-transfer chromatography of lipids, *e.g.*, ion exchangers in the Ag^+ form[59,60,62,126].

B. Class separations and individual substance separations

Group or class separations predominate in work on lipids[1–3,5,6,18,68,93,127–131]. Traditionally, these separations are carried out by adsorption on silica gel, followed by stepwise or gradient elution with a number of solvents, frequently more than two solvents (Table 1). For certain analytical purposes, a polar solvent front has been used to displace several classes of lipids from an adsorption column[118]. Reversed-phase partition has also been used for class separations[127,132]. Separations of hydrocarbon, fatty acid methyl esters and triglyceride classes are sometimes carried out by isocratic elution[74,133]. Molecular size separations by GPC belong in a similar category. A GPC–adsorption separation has been proposed by Brooks and Keates[108] that gives three groups, *viz.*, in order of elution, high-molecular-weight materials, a fraction suitable for direct GLC analysis, and polar materials.

A special type of separation that may occasionally be applicable to lipids is the micelle separation. Lipids may exist as micelles in a polar solvent (*e.g.*, water) and as "reversed" micelles in hydrocarbon solvents. These micelles can be separated from other lipids by GPC, where micelles are excluded because of their size[134], and by adsorption chromatography on silica gel in hydrocarbon solvents. In that case the micelles of phospholipids are eluted with the void volume while triglycerides are retarded by adsorption[135].

Another potential situation for a group separation is the separation of branched or alicyclic lipids from linear lipids, as in urea columns.

For separations of lipid classes, quantitation is a major problem.

For the separation of closely related individual lipids, within a class, by partition or reversed-phase LC, gradients are often not necessary, so that readily available commercial HPLC equipment can be used (*e.g.*, isocratic elution with RI detection). However, Lindqvist *et al.*[136] achieved excellent preparative separations of milk fat triglycerides by carbon number, using gradient elution reversed-phase partition with transport flame ionization detection. Up to 10,000 plates per metre have been achieved by partition on methylated Sephadex[104,137], but the separations are slow and pumps cannot be used with the compressible gels. Homologous K vitamins[137], individual fatty acids[110,138–140], fatty acid methyl esters[110,132,141–143], fatty alcohols[144], cholesterol esters[132,145] and homologous series of triglycerides[73,136,143,146–150] have been separated by one or more modes of LC.

4. APPLICATIONS

A. Biological lipids

(a) Surface lipids

Surface lipids are often typical of both plant and animal species and can be used for taxonomic classification. Their lipid class compositions may vary considerably, and some unique features have been found in sebum samples (hydrocarbons, waxes, squalene, diol lipids, long-chain alcohols, odd-numbered, branched and Δ^6-unsaturated acids, etc.). Both the medical profession (dermatology; disorders such as acne and psoriasis) and industry (*e.g.*, skin and hair cosmetics) are interested in sebum analyses.

There are dozens of papers dealing with analytical methods and quantitation of classes of lipids in the human skin surface fat by TLC. The first transport FIDs for column LC were actually designed for analyses of lipid classes in human sebum[18,19] and of the vernix caseosa of the human newborn[19,130], which contains a class of lipids (diol lipids) not found in the adult.

There have been a number of reports from Haahti's group on LC separations of both human and animal skin surface sebum samples, using transport FID detection[18,19,103,130,151,152]. Recently, Aitzetmüller and Koch[153] investigated the LC of sebum samples using 20-min gradient elution programmes.

Wool alcohols have been analyzed by Young[154] using stepwise gradients and transport FID detection.

(b) Blood, serum and organ lipids

Many workers have investigated the LC of blood serum or plasma lipid extracts (Table 1) and the LC of lipids of whole blood cells[112] (Fig. 1).

A total lipid extract of the human aorta was analyzed by Anderson *et al.*[158] using benzene elution from a cholanyl-Sephadex LH-20 column and a moving-wire detector.

One of the earliest applications of a transport FID was the separation of human

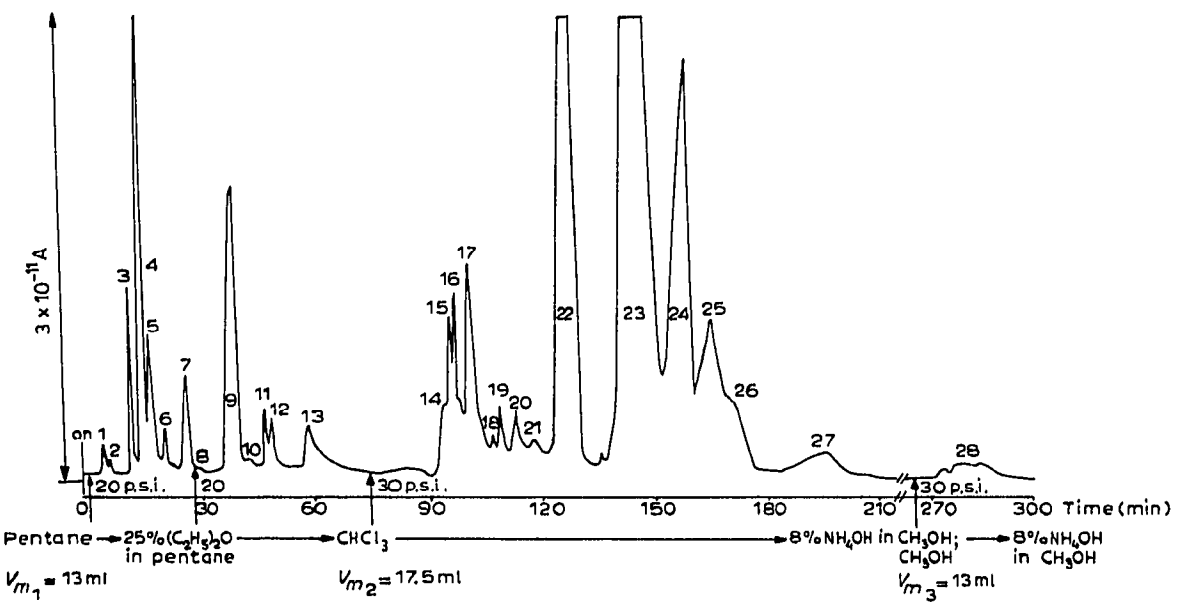

Fig. 1. Chromatogram of lipids of red blood cells of the rat. 4 = Triglycerides; 9 = cholesterol; 22 = phosphatidylethanolamine; 23 = phosphatidylcholine; other elution bands unidentified. From Stolyhwo and Privett[112].

serum sterol esters by degree of unsaturation, using silver nitrate adsorption chromatography plus gradient elution[18].

Lang[159] demonstrated the GPC of glycerides isolated from the liver and other organs. Rat liver lipids[129] and kidney lipids[120] have also been analyzed by gradient elution from a silica gel column, using a transport FID, and the unsaponifiable portion of rat liver lipids was analyzed by LC plus UV detection[67].

Phospholipids from rat tissues, serum[160], egg yolk[161] and human bile[162] have been analyzed by HPLC. Conditions for the separation of phosphatidylcholine and sphingomyelin by HPLC were investigated by Rainey *et al.*[163] using a transport FID. Almé *et al.*[125] analyzed phosphatidylcholines by reversed-phase chromatography.

The separation principles applicable to brain lipids have been discussed by Rouser[6]. Other workers have investigated the liquid chromatography of acidic brain phospholipids[123] and of other brain lipids[120,164]. Stolyhwo and Privett[112] analyzed beef brain cerebroside fractions by gradient elution LC with a transport FID of improved design. The analysis of (benzoylated) human tissue, plasma and urine glycolipids[85] and ceramides[84] by HPLC plus UV detection has been described (Fig. 2).

Bile acids can be separated by using adsorption systems similar to the common TLC separations, and detected by a transport FID[74]. Reversed-phase partition has also been tried[165].

For steroids and vitamins in blood, tissues and urine, see Sections 4A(c) and 4B(b).

(c) Steroids, prostaglandins and pheromones

Steroids, particularly those which absorb in the UV region, have been the sub-

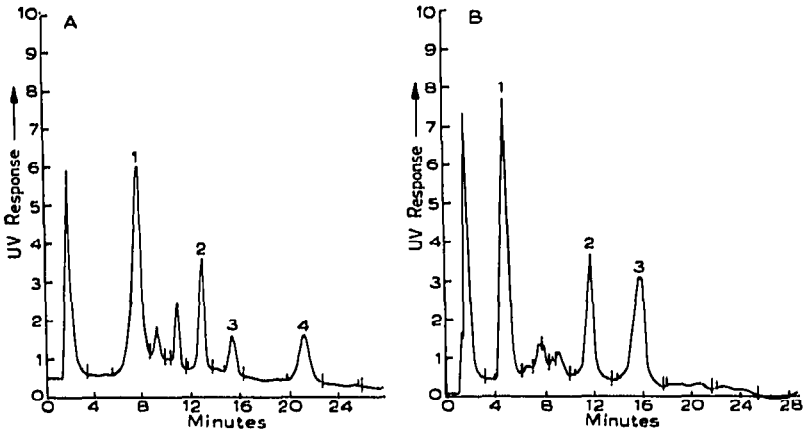

Fig. 2. HPLC analysis of benzoylated plasma glycosphingolipids. A = From normal; B = from Fabry's disease plasma glycolipid fractions. 1, 2, 3, 4 = Mono-, di-, tri- and tetrahexosylceramides, respectively. From Evans and McCluer[85].

ject of a very large number of HPLC publications, mostly of biomedical or pharmaceutical interest (Table 1). These papers, which are of only marginal interest here, are not completely covered in this review. All of the major instrument companies have issued applications literature for the HPLC analysis of steroids.

The analysis of steroids and other low-molecular-weight substances from the unsaponifiable portion of a lipid extract is one of those borderline cases where both GLC and HPLC are frequently used. GLC is still the method of choice for the more complicated mixtures of biological origin, but HPLC is frequently preferred for fast routine pharmaceutical tablet analysis, where only a few substances are present in large amounts. However, in some instances, HPLC is preferred over GLC for the analysis of certain steroids in biological fluids because of time saved during the pre-treatment of the sample (*e.g.*, the extraction procedure) when only one compound of medical interest is to be determined and large numbers of routine analyses are to be run[155]. Both blood and tissue steroids[78,79,155,166] and urine steroids[78,79,167–169] have been analyzed by HPLC.

UV, RI and fluorescence detectors are most frequently used for the LC of steroids of medical interest (Table 1). For some of the non-UV-absorbing steroids, derivatization with a UV-absorbing reagent has been tried[79–81]. The early work of Vestergaard and co-workers[88,89] on the separation of urinary 17-keto-steroids by gradient elution from alumina columns should also be mentioned.

Transport FIDs have also been used for steroid analyses[129,170–173] and for the detection of oxidation products in cholesterol[174]. Cholesterol and cholesteryl esters are often determined in surface lipids, blood and organ lipids and animal fats, and phytosterols (*e.g.*, sitosterol, stigmasterol and campesterol) and their esters in edible fats of plant origin. A number of workers have separated C_{27}–C_{29} sterols by carbon number, using reversed-phase partition LC[110,175,176]. Cholesteryl esters have been separated by chain length[132,145] and by degree of unsaturation[18,154].

Prostaglandins have also been the subject of a few recent LC publications[57,59,73,177,178], their UV absorbance[177] or that of suitable derivatives being used

for detection. Their separation, by degree of unsaturation, on Ag^+-impregnated columns has been reported[59,73]. HPLC separations of prostaglandins on Corasil II columns with RI detection were described by Andersen and Leovey[57]. RI detection was also used by Weinshenker and Longwell[178].

Lipid pheromones have also been analyzed by HPLC by degree of unsaturation[62], and there are reports on the HPLC of insect juvenile hormones[73,81,179–182].

(d) Plant lipids and other biological lipids

Carotenoids are frequently found in the unsaponifiable portion of plant lipids, where they occur both in the free form and esterified with long-chain fatty acids.

Stewart and Wheaton[72] described the separation of carotenes and xanthophylls by HPLC with gradient elution on slurry-packed magnesium oxide and zinc carbonate columns.

Elution data for a large number of biological lipids on hydroxyalkoxypropyl-Sephadex were given by Brooks and Keates[108].

The lipid composition of developing soya beans was analyzed by Privett *et al.*[116]. By gradient elution and the use of a transport FID, 29 fractions were found (Fig. 3).

Davies and Mercer[71] investigated methods for the analysis of unsaponifiable lipids in plants, and Young[154] analyzed leaf lipids.

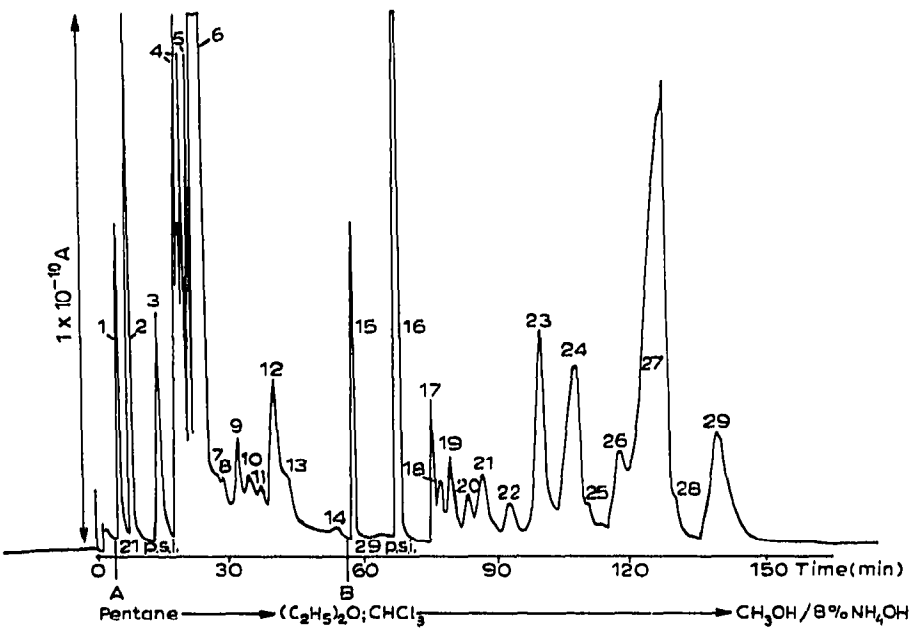

Fig. 3. Analytical liquid chromatography of lipids of immature soya beans. Peaks: 1–3 = hydrocarbons, waxes, pigments and unknowns; 4, 5 = sterol esters; 6 = triglycerides; 7–11, 14, 18, 20, 25, 28 = unknowns; 12 = sterols; 13 = free fatty acids; 15 = esterified sterol glucosides; 16 = monogalactosyl diglycerides; 17 = sterol glucosides; 19 = cerebroside; 21 = phosphatidylglycerol; 22 = digalactosyl diglyceride; 23 = phosphatidylethanolamine; 24 = phosphatidylinositol; 26 = phosphatidylcholine; 27 = phosphatidic acid; 29 = lysophosphatidylcholine and other very polar lipids. From Privett *et al.*[116].

B. Food lipids

(a) Edible fats and products of fat alteration

Edible fats are sometimes stabilized by the addition of certain antioxidants. These, and the natural tocopherols, can be detected by their UV absorption in the LC of both a fat extract[65], the intact fat[73,75] and the unsaponifiable portion of the fat[66,67].

Natural edible oils were investigated by gradient elution adsorption LC[129] and by GPC[148]. Butterfat triglycerides were separated according to their carbon numbers by reversed-phase partition LC[136].

Dimeric triglycerides, which are sometimes formed in food lipids by oxidative or thermal dimerization, are conveniently detected by GPC[69,159,183–188] and adsorption LC[69,189] (Fig. 4). Graille *et al.*[190] analyzed dimeric methyl esters using adsorption LC with a transport FID.

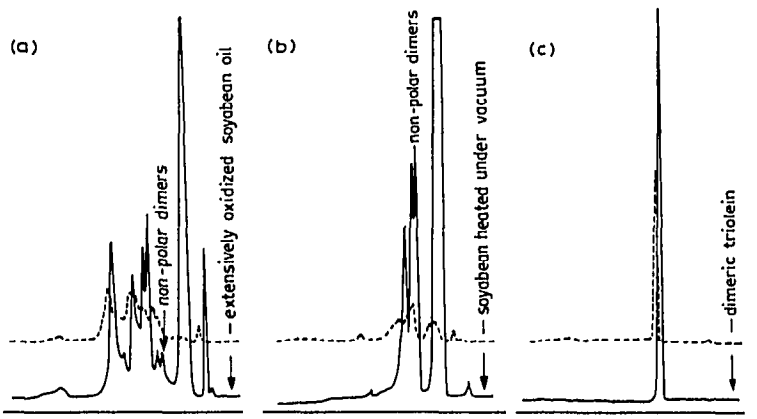

Fig. 4. Gradient elution liquid chromatograms of model oils. (a) Soya bean oil that had been heated under an oxygen atmosphere; (b) soya bean oil that had been heated under vacuum; (c) dimeric triolein (test substance). – – –, UV, 254 nm; ——, wire. From Aitzetmüller[69].

Adsorption LC was used for the stepwise displacement of polar materials (hydroxy triglycerides, diglycerides and oxidized polymers)[191], of a "total artefacts" peak (which included the non-polar dimeric triglycerides)[68,118], and for the analysis of the unchanged triglyceride content[106] of used frying oils. Gradient elution from silica gel columns[68,69,189,192] may lead to "fingerprint" chromatograms that give a more detailed picture of the artefact composition in a used oil.

If partial glycerides are present, they can be analyzed by GPC[66,185], adsorption LC[129,189] and partition and reversed-phase partition LC[127,175] (see also Table 1).

Oxidation products in fats may frequently contain conjugated double bonds that can be detected in LC by their UV absorption[68,69]. Chan and Prescott separated the isomeric 9- and 13-hydroperoxides of methyl linoleate[70] and of free linoleic acid[193].

Changes in the unsaponifiable portion of an edible fat[66] may be indicators of adulteration, refining and inter-esterification, and are a potential field of future applications of HPLC, although GLC may be preferred.

Another potential future application of HPLC is the analysis of triglycerides by degree of unsaturation, which has no counterpart in GLC. No such publications on the analysis of triglycerides have appeared yet, but the technique works well with unsaturated fatty acid methyl esters[74,103,154].

For vitamins in edible fats, see the next section.

(b) Lipid-soluble vitamins

Applications literature for the HPLC analysis of lipid-soluble vitamins can be obtained from all major instrument companies. As with steroids, the lipid-soluble vitamins have been the subject of a large number of publications (Table 1), probably because they can all be conveniently detected with a UV detector. Again, pharmaceutical tablet analysis[194–197] for routine quality control is the main application. In contrast, analyses involving actual food products are rather scarce[198–202].

Homologous K vitamins have been separated by LLC on methylated Sephadex[137]. The effluent was monitored by one of the early versions of a transport FID, although UV detection is also applicable in this instance.

The GPC of tocopherols and tocoquinone oligomers in the unsaponifiable portion of edible fats was discussed briefly by Pokorny *et al.*[66]. The GPC of cod liver oil and the reversed-phase HPLC analysis of vitamin A in a preparative GPC fraction of this oil were carried out by Williams *et al.*[194]. V.d. Weerdhof *et al.*[87], Van Niekerk[90] and Thompson *et al.*[91] used fluorimetric detection for determinations of vitamin A and tocopherols, respectively. Scott and Kucera[174,203] analyzed tocopherol oil distillates with a moving-wire detector. Vitamin A and D in fish liver oil have been analyzed by Young[154], and vitamin A in margarine and eggs by Bell[202].

Ubiquinone, α-tocopherylquinone, α-tocopherol and retinol were determined in a chloroform–Sephadex system by switching the wavelength of the UV detector from 277 to 292 and finally to 325 nm as the peaks were eluted[67].

Vitamins and related compounds in biological samples were analyzed by Ito *et al.*[204] and by Stacewicz-Sapuncakis *et al.*[205].

(c) Food emulsifiers, "lecithin" and other food lipids

Baur[206] predicted that HPLC might become the method of choice for the difficult analysis of food emulsifiers. Most of these substances are UV-transparent (*e.g.*, they contain only saturated fatty acids) and require gradient elution, so that the general lipid detection problem discussed in Sections 2A and 2B is evident again. It is therefore not surprising that so little work has been carried out in order to follow up Baur's[206] prediction.

Sorbitan sesquioleate was analyzed by Young[154] by gradient elution from a silicic acid column, plus transport FID detection. Aitzetmüller[119] has shown that fingerprint chromatograms for a number of food emulsifiers can be obtained by gradient elution LC from Porasil columns. Fig. 5 shows the analysis of lactylated and acetylated glycerides in food products.

In commercial soya bean "lecithin", 19 fractions were found by Erdahl *et al.*[117] using a high-resolution multi-solvent gradient elution system with a transport FID. An applications note from Varian[207] also describes the HPLC of lecithin from soya; two fractions were found with a UV detector set at 210 nm. GPC has been used

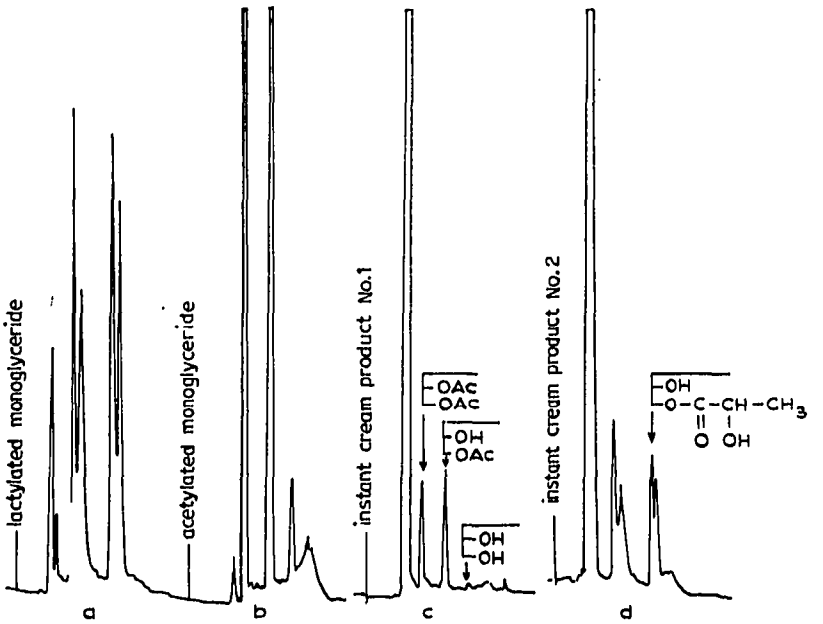

Fig. 5. Analysis of lactylated and acetylated monoglycerides in food products. From Aitzetmüller[119].

to investigate egg lecithin[208]. Arvidson[209] analyzed egg-yolk lecithins on lipophilic Sephadex columns with RI detection.

C. Technical fats and oleochemicals

(a) Technical dimeric fatty acids

Technical monomeric, dimeric and trimeric fatty acids, which are raw materials for polymers, can be conveniently analyzed by GPC using RI detectors[210–213]. They have also been separated by adsorption LC on columns of Porasil A[214]. The methyl esters of dimeric fatty acids can also be analyzed by GPC on Sephadex LH-20[186,188,210,215], and dimeric and trimeric triglycerides by GPC on Bio-Beads in benzene[215]. Partition chromatography is also frequently used[98,154]. Non-polar dimeric triglycerides can be separated from monomeric triglycerides by adsorption LC on Porasil[69,189].

(b) Technical glycerides, epoxidized glycerides and alkyd resins

Technical glycerides, *e.g.*, castor oil[148], vernonia oil[68], partial glycerides[129,216], oxidized glycerides[69,185,189,215] and drying oils[148], have also been analyzed by LC. Epoxidized soya bean oil can be analyzed according to the number of oxirane oxygen atoms per triglyceride molecule by gradient elution adsorption chromatography on Porasil[74].

Vinylated vegetable oils have been analyzed by GPC[212], and styrene derivatives of fatty acids by adsorption LC plus UV detection[199].

Alkyd resins have been analyzed by GPC for their molecular weight distribution[212,217–220] and for their contents of residual mono-, di- and triglycerides[217,218].

(c) Waxes

Hillman[221] has analyzed waxes by GPC. Fast adsorption LC is also applicable to the analysis of wax mixtures for their contents of hydrocarbon, monoester or diester waxes and other classes of lipids. Gradient elution with a transport FID may be required as most waxes do not absorb in the UV region. Popl and Havel[121] have analyzed fractions of montana wax in this manner.

Ellingboe *et al.*[175] have separated monoester waxes by reversed-phase partition.

(d) Technical emulsifiers and detergents based on fatty acid and fatty alcohol derivatives

As with food emulsifiers, HPLC should be the method of the future for the analysis of ethoxylated fatty alcohols and fatty acids[119], and of ethoxylated partial glycerides. In view of the importance of the detergents industry, surprisingly few attempts have been made so far to document the applicability of HPLC to the analysis of aliphatic (*i.e.*, non-UV-absorbing) technical surfactants[101,149,222,223]. Application notes from instrument companies are scarce, and a few other publications deal with UV-absorbing derivatives.

Hindley[224] analyzed the contents of unsulphated material in fatty alcohol sulphates and alkyl ether sulphates.

Adsorption, partition and reversed-phase partition LC are applicable to non-ionic ethoxylates, while ion-exchange LC is suitable for cationic and anionic derivatives[225]. Homologous fatty alcohols have been analyzed by partition[222,226] and reversed-phase partition LC[144,158,226].

The lack of suitable detectors for the gradient elution LC of UV-transparent surfactants is probably the main reason why the potential of HPLC in this field has not yet been fully recognized.

Another new development may permit the UV detection of ionic surfactants paired with UV-absorbing counterions, in ion-pair partition chromatography.

(e) Other oleochemicals

Among the other oleochemicals that have occasionally been analyzed by HPLC are fatty acid amides[73,227,228] (erucamide, stearamide, oleamide, etc.), and compounds such as distearyl- and dilauryl thiodipropionates[73], which are used as polymer additives.

Oligomeric fatty alcohols[210] and ethers[227] have been analyzed bGy PC. Partial phosphate esters of a long-chain alcohol were analyzed, after methylation, by GPC on Sephadex LH-20[146].

Steel preservatives, rolling and drilling oils and engine oils, etc., sometimes contain triglycerides (*e.g.*, castor oil) and detergents. They can be analyzed by GPC[212] or by gradient elution adsorption LC[74].

Polar fatty acids and their methyl esters (*e.g.*, methyl ricinoleate, epoxystearate, ketooleate, etc.) can be investigated by LLC on "factice" columns[20], or by gradient elution adsorption LC[229]. Technical conjugated octadienoic fatty acids were separated by resin (Ag^+) LC[60]. Isocyanates derived from fatty acids were analyzed by Washburne and Peterson[230].

Resin acid methyl esters can be separated from fatty acid methyl esters by LC in diethyl ether on polystyrene[139], the former esters being eluted later than the latter.

TABLE 1

APPLICATIONS OF THE LIQUID CHROMATOGRAPHY OF LIPIDS

Books, reviews and papers of a purely technical nature, and some of the more conventional chromatography publications, which may have been quoted in the text have been omitted from this table. On the other hand, the table contains some additional references that have not been mentioned in the text. Most of the column headings are self-explanatory, but some remarks are given as footnotes.

Reference	*Experimental*																																		
	LC modes, column filling															*Technical and theoretical details*																			
	Adsorption						*Partition*					*GPC*				*Detection*														*Solvent transport*					
	Silica	*Alumina*	*Complex (Ag⁺, etc.)*[a]	*Other*[b]	*Pellicular materials*	*Ion exchangers*	*Bonded phases*	*Reversed phase*	*Normal LLC*	*Sephadex + derivatives*	*Sephadex*	*Polystyrene*	*Other*	*Ultraviolet (UV)*	*Refractometer (RI)*	*Transport FID*	*Other*	*Derivatization*	*Quantitation*	*Theory (polarities, "α")*[c]	*Technical papers*	*Temperature + other parameters*[d]	*Pump used*	*Gas 1p on solvent*	*Isocratic*	*Gradient/steps*	*More than 2 solvents*[e]								
6	×			×		×			×					×				×		×						×	×								
18	×		×													×					×						×	×								
19	×		×													×					×						×	×								
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81					×			×	×					×	×			×																	

Separation type		Applications																								Reference
		Biological lipids															Food lipids						Technical lipids			
		Sample origin				Lipid class[j]																				
Group or class separation	Individual substance separation	Blood, biological fluids	Organs, tissues	Sebum, skin, hair	Other biological/plant lipids	Steroids I (pharmaceuticals)[f]	Steroids II (fat)[g]	Prostaglandins, etc.[h]	Phospholipids	Other polar lipids	R-OH, R-CHO, R-CO-R, etc.[i]	Hydrocarbons, squalene	Triglycerides	Partial glycerides	Free fatty acids	Fatty acid esters	Edible oils, fats	Unsaponifiable	Lipid vitamins	Oxidation products	Dimeric products	Emulsifiers	Detergents	Paints, waxes, tech. glyc.	Other oleochemicals	
×	×	×	×		×	×	×		×	×																6
×	×	×		×			×				×	×	×			×										18
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	×	×				×																				79
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	×					×		×																		81

(Continued on p. 250/251)

TABLE 1 *(continued)*

Reference	*Experimental*																																				
	LC modes, column filling															*Technical and theoretical details*																					
	Adsorption					*Partition*						*GPC*			*Detection*														*Solvent transport*								
	Silica	*Alumina*	*Complex (Ag^+, etc.)*[a]	*Other*[b]	*Pellicular materials*	*Ion exchangers*	*Bonded phases*	*Reversed phase*	*Normal LLC*	*Sephadex + derivatives*	*Sephadex*	*Polystyrene*	*Other*	*Ultraviolet (UV)*	*Refractometer (RI)*	*Transport FID*	*Other*	*Derivatization*	*Quantitation*	*Theory (polarities, "ε")*[c]	*Technical papers*	*Temperature + other parameters*[d]	*Pump used*	*Gas Δp on solvent*	*Isocratic*	*Gradient/steps*	*More than 2 solvents*[e]
82					×			×						×	×			×	×				×		×												
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101					×		×	×								×	×				×		×														
103	×		×													×	×		×		×		×			×	×										
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107	×															×			×		×		×	×			×	×									
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112	×				×											×	×			×	×			×			×	×									
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117	×				×											×								×			×	×									
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120	×		×		×				×					×		×							×		×		×	×									
121	×															×							×				×										
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Separation type		*Applications* — *Biological lipids* — *Sample origin*				*Lipid class*[j]											*Food lipids*					*Technical lipids*				*Reference*
Group or class separation	*Individual substance separation*	*Blood, biological fluids*	*Organs, tissues*	*Sebum, skin, hair*	*Other biological/plant lipids*	*Steroids I (pharmaceuticals)*[f]	*Steroids II (fat)*[g]	*Prostaglandins, etc.*[h]	*Phospholipids*	*Other polar lipids*	*R-OH, R-CHO, R-CO-R, etc.*[i]	*Hydrocarbons, squalene*	*Triglycerides*	*Partial glycerides*	*Free fatty acids*	*Fatty acid esters*	*Edible oils, fats*	*Unsaponifiable*	*Lipid vitamins*	*Oxidation products*	*Dimeric products*	*Emulsifiers*	*Detergents*	*Paints, waxes, tech. glyc.*	*Other oleochemicals*	
	×					×	×																			82
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	×						×								×								×			101
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×					×				×																	123
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	×								×																	125

(Continued on p. 252/253)

TABLE 1 *(continued)*

Reference	Experimental: LC modes, column filling — Adsorption: Silica	Alumina	Complex (Ag^+, etc.)[a]	Other[b]	Partition: Pellicular materials	Ion exchangers	Bonded phases	Reversed phase	Normal LLC	GPC: Sephadex + derivatives	Sephadex	Polystyrene	Other	Detection: Ultraviolet (UV)	Refractometer (RI)	Transport FID	Other	Derivatization	Technical and theoretical details: Quantitation	Theory (polarities, "u")[c]	Technical papers	Temperature + other parameters[d]	Solvent transport: Pump used	Gas Ip on solvent	Isocratic	Gradient/steps	More than 2 solvents[e]
127								×								×			×	×		×	×		×		
128	×															×			×							×	×
129	×															×			×	×	×		×			×	
130	×															×										×	
131	×																				×					×	×
132								×							×					×	×				×		
133	×							×								×			×		×				×		
136								×		×						×					×		×			×	
137									×	×						×								×	×		
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139				×								×			×										×		
140													×		×						×				×		
141								×							×						×				×		
142	×				×	×	×	×						×							×				×		
143								×							×										×		
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150	×								×			×		×	×						×		×		×		
151	×															×					×		×				
152	×										×					×							×		×		
153	×															×			×		×		×				
154	×		×			×		×	×							×			×	×	×	×	×	×	×		×
155	×								×					×					×				×				
156	×							×						×					×				×				
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159													×		×								×		×		
161	×															×											
162	×																						×			×	×
164	×																			×	×	×	×			×	

Separation type	Group or class separation	Individual substance separation	Blood, biological fluids	Organs, tissues	Sebum, skin, hair	Other biological/plant lipids	Steroids I (pharmaceuticals)[f]	Steroids II (fat)[g]	Prostaglandins, etc.[h]	Phospholipids	Other polar lipids	R-OH, R-CHO, R-CO-R, etc.[i]	Hydrocarbons, squalene	Triglycerides	Partial glycerides	Free fatty acids	Fatty acid esters	Edible oils, fats	Unsaponifiable	Lipid vitamins	Oxidation products	Dimeric products	Emulsifiers	Detergents	Paints, waxes, tech. glyc.	Other oleochemicals	Reference
			Applications																								
			Biological lipids																							*Food lipids*		*Technical lipids*	
			Sample origin				*Lipid class*[j]																				
	×			×																×							127
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	×	×		×			×	×		×								×								129
	×	×			×			×		×																	130
	×	×	×					×		×																	131
	×	×	×					×																			132
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	×	×	×																								136
	×	×	×																								137
	×	×	×																								138
	×	×	×																								139
	×	×	×																								140
	×	×	×																								141
	×	×	×																								142
	×	×	×																								143
	×	×	×																								144
	×	×	×																								145
	×	×	×																								146
	×	×	×																								147
	×	×	×																								148
	×	×	×																								149
	×	×	×																								150
	×	×	×																								151
	×	×	×																								152
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	×	×	×																								155
	×	×	×																								156
	×	×	×																								157
	×	×	×																								158
	×	×	×																								159
	×	×	×																								161
	×	×	×																								162
	×	×	×																								164

(Continued on p. 254/255)

TABLE 1 *(continued)*

Reference	Silica	Alumina	Complex (Ag^+, etc.)[a]	Other[b]	Pellicular materials	Ion exchangers	Bonded phases	Reversed phase	Normal LLC	Sephadex + derivatives	Sephadex	Polystyrene	Other	Ultraviolet (UV)	Refractometer (RI)	Transport FID	Other	Derivatization	Quantitation	Theory (polarities, "a")[c]	Technical papers	Temperature + other parameters[d]	Pump used	Gas Δp on solvent	Isocratic	Gradient/steps	More than 2 solvents[e]
	Experimental																										
	LC modes, column filling													*Technical and theoretical details*													
	Adsorption							*Partition*			*GPC*			*Detection*									*Solvent transport*				
165								×									×				×				×		
166									×					×					×	×		×	×		×		
167	×				×									×					×				×		×		
168									×					×					×	×	×		×		×		
169									×					×									×			×	
170	×															×										×	
171	×															×				×	×	×	×			×	
172	×				×									×		×			×	×	×	×	×			×	
173				×						×						×				×		×	×	×	×		
174	×															×				×			×			×	×
175								×	×	×				×		×				×					×		
176								×		×													×		×		
177	×													×				×	×					×	×		
178	×														×				×				×		×		
179	×				×									×									×		×		
180	×				×									×									×		×		
181						×		×											×				×			×	
182								×						×						×			×		×	×	
183												×			×				×	×	×		×		×		
185												×			×				×	×	×		×		×		
186	×										×	×			×	×							×	×	×	×	×
187	×											×			×	×							×		×	×	×
188											×	×			×				×					×	×		
189	×											×			×	×							×	×	×	×	×
190	×															×							×		×		
191	×															×			×					×		×	×
192	×										×	×			×	×							×	×		×	×
194					×			×				×		×	×								×		×	×	
195	×				×					×				×					×	×			×		×		
196	×													×					×				×		×		
197	×	×			×			×						×					×				×		×		
198		×			×									×									×		×		
199	×													×					×				×		×		
200	×													×									×		×		
201	×				×			×						×									×		×	×	

Separation type		Applications: Biological lipids — Sample origin				Lipid class[l]												Food lipids								Technical lipids		Reference
Group or class separation	Individual substance separation	Blood, biological fluids	Organs, tissues	Sebum, skin, hair	Other biological/plant lipids	Steroids I (pharmaceuticals)[f]	Steroids II (fat)[g]	Prostaglandins, etc.[h]	Phospholipids	Other polar lipids	R-OH, R-CHO, R-CO-R, etc.[i]	Hydrocarbons, squalene	Triglycerides	Partial glycerides	Free fatty acids	Fatty acid esters	Edible oils, fats	Unsaponifiable	Lipid vitamins	Oxidation products	Dimeric products	Emulsifiers	Detergents	Paints, waxes, tech. glyc.	Other oleochemicals	
	×						×																			165
	×	×				×																				166
	×	×				×																				167
	×	×				×																				168
	×		×			×																				169
	×					×																				170
	×					×							×													171
	×					×																				172
×	×					×	×						×													173
×	×						×					×				×			×							174
	×				×	×	×					×	×	×		×								×		175
	×						×																			176
	×		×					×																		177
	×							×																		178
	×							×																		179
	×							×																		180
	×				×			×																		181
	×							×																		182
×													×	×	×		×				×					183
×													×	×	×		×			×	×					185
×													×			×	×			×	×					186
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×																×				×	×					190
×							×					×	×				×			×						191
×													×	×		×	×			×	×					192
	×		×										×				×		×							194
	×																		×							195
	×																		×							196
	×																		×							197
	×																		×							198
	×	×				×											×		×						×	199
	×																×	×	×							200
	×					×													×							201

(Continued on p. 256/257)

TABLE 1 *(continued)*

Reference	Experimental: LC modes, column filling: Adsorption: Silica	Alumina	Complex (Ag^+, etc.)[a]	Other[b]	Partition: Pellicular materials	Ion exchangers	Bonded phases	Reversed phase	Normal LLC	Sephadex + derivatives	GPC: Sephadex	Polystyrene	Other	Detection: Ultraviolet (UV)	Refractometer (RI)	Transport FID	Other	Derivatization	Technical and theoretical details: Quantitation	Theory (polarities, "α")[c]	Technical papers	Temperature + other parameters[d]	Solvent transport: Pump used	Gas ∆p on solvent	Isocratic	Gradient/steps	More than 2 solvents[e]
202									×	×				×											×		
204									×	×				×										×	×		
205	×				×									×									×		×		
207	×				×		×							×									×		×	×	
208													×		×										×		
209								×		×					×							×			×		
210											×				×				×	×					×		
211											×	×			×				×	×			×		×		
212												×			×				×	×	×		×		×		
213											×	×			×				×			×	×		×		
215											×					×								×	×		
216	×						×									×							×		×	×	
217												×			×								×		×		
218												×			×				×	×	×		×		×		
220												×			×						×		×		×		
221												×			×								×		×		
222								×	×						×								×		×		
223											×				×								×		×		
224						×										×							×	×	×		
225						×								×									×		×		
226				×				×		×	×					×				×			×		×		
227												×			×						×				×		
229	×															×					×			×		×	
230									×						×									×			
231	×															×				×		×	×			×	
232	×															×				×		×	×				
233	×															×				×		×	×				
234												×			×												
235	×				×		×	×						×	×				×				×		×	×	
236	×													×		×							×		×		
237								×															×			×	
238	×						×	×						×									×				
239	×													×									×				
240					×		×	×	×					×					×				×				
241									×					×									×				

[a] Complex (Ag^+, etc.). [b] Other. [c] Theory (polarities, "α"). [d] Temperature + other parameters. [e] More than 2 solvents.

Separation type		Applications																								
		Biological lipids															Food lipids						Technical lipids			Reference
		Sample origin				Lipid class[j]																				
Group or class separation	Individual substance separation	Blood, biological fluids	Organs, tissues	Sebum, skin, hair	Other biological/plant lipids	Steroids I (pharmaceuticals)[f]	Steroids II (fat)[g]	Prostaglandins, etc.[h]	Phospholipids	Other polar lipids	R-OH, R-CHO, R-CO-R, etc.[i]	Hydrocarbons, squalene	Triglycerides	Partial glycerides	Free fatty acids	Fatty acid esters	Edible oils, fats	Unsaponifiable	Lipid vitamins	Oxidation products	Dimeric products	Emulsifiers	Detergents	Paints, waxes, tech. glyc.	Other oleochemicals	
	×	×	×		×												×		×							202
	×	×	×																×							204
	×		×																×							205
	×				×				×										×			×				207
×					×				×				×			×										208
×			×						×																	209
×															×	×					×				×	210
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	×					×													×							238
	×					×													×							239
	×					×																				240
	×					×																				241

(Continued on p. 258/259)

TABLE I *(continued)*

Reference	Experimental																														
	LC modes, column filling													Technical and theoretical details																	
	Adsorption				Partition					GPC				Detection												Solvent transport					
	Silica	Alumina	Complex (Ag^+, etc.)[a]	Other[b]	Pellicular materials	Ion exchangers	Bonded phases	Reversed phase	Normal LLC	Sephadex + derivatives	Sephadex	Polystyrene	Other	Ultraviolet (UV)	Refractometer (RI)	Transport FID	Other	Derivatization	Quantitation	Theory (polarities, "α")[c]	Technical papers	Temperature + other parameters[d]	Pump used	Gas bp on solvent	Isocratic	Gradient/steps	More than 2 solvents[e]					
242					×				×					×					×													
243							×		×					×					×		×										
244									×					×	×					×	×										
245												×			×					×		×									
246	×	×												×																	
247								×								×					×										
248					×		×	×						×																	
249	×													×		×															
250	×													×				×	×												
251	×													×					×												
252					×		×	×							×																
253					×	×								×					×												
254							×	×						×				×	×												
255					×		×	×							×				×	×											
256	×						×	×						×																	

[a] Silver nitrate-impregnated silica and other charge-transfer chromatography media, *e.g.*, rhodium derivatives.

[b] For example: MgO, $ZnCO_3$, adsorption on a gel matrix, boric acid-impregnated silica.

[c] Discussion of sample and solvent polarities, sorbent and solvent selectivity, separation factors (α), etc.

[d] Discussion of the influence of temperature, pH, ionic strength, flow-rates, column dimensions, etc.

[e] Gradient systems using more than two solvent reservoirs.

5. QUANTITATION

Quantitation in the LC of lipids is complicated by the fact that group or class elution predominates. When a class of lipids is eluted to give a single liquid chromatographic peak, this peak will contain a number of compounds with similar structures. However, these compounds may still differ in the functional groups that they contain, *e.g.*, in the degree of unsaturation and in the presence or absence of conjugation. Such structural features may influence both the refractive index and the UV absorption properties of some of the lipid compounds that are eluted together in one peak.

UV detectors may indicate only the UV-absorbing fraction within the peak of

Separation type	Applications																													Reference
	Biological lipids													Food lipids													Technical lipids			
	Sample origin					Lipid class[j]																								
Group or class separation	Individual substance separation	Blood, biological fluids	Organs, tissues	Sebum, skin, hair	Other biological/plant lipids	Steroids I (pharmaceuticals)[f]	Steroids II (fat)[g]	Prostaglandins, etc.[h]	Phospholipids	Other polar lipids	R-OH, R-CHO, R-CO-R, etc.[i]	Hydrocarbons, squalene	Triglycerides	Partial glycerides	Free fatty acids	Fatty acid esters	Edible oils, fats	Unsaponifiable	Lipid vitamins	Oxidation products	Dimeric products	Emulsifiers	Detergents	Paints, waxes, tech. glyc.	Other oleochemicals	
	×					×																								242
	×					×																								243
	×					×	×																							244
×												×			×															245
	×					×													×											246
	×														×															247
	×																		×											248
	×											×				×			×											249
×			×						×																					250
×			×						×																					251
	×												×		×	×														252
	×							×																						253
	×							×																						254
	×															×														255
	×							×					×				×							×						256

[f] Steroids of primarily pharmaceutical or biomedical interest (*e.g.*, tablet constituents, hormones). Mostly UV-absorbing.

[g] Steroids typical for the unsaponifiable portion of fats, phytosterols and cholesterol and their esters. Bile acids, etc. Mostly non-UV-absorbing.

[h] Prostaglandins, pheromones, insect moulting hormones and related lipids.

[i] Fatty alcohols, aldehydes, ethers, ketones, lactones, etc.

[j] Lipid class entries also for "food" and "technical" lipids.

a class of lipids, *e.g.*, the conjugated diene portion. Highly unsaturated lipids may have a different refractive index than their saturated analogues. The same is true for diglycerides and triglycerides, and may cause problems if "neutral lipids" are eluted together, *e.g.*, in front of a phospholipid separation.

If UV or RI detectors are used in order to quantitate such composite "lipid class peaks", errors may result if the composition of the peak differs from sample to sample, or from that of a standard class of lipids that was used to calibrate the instrument.

For the quantitation of classes of lipids, the ideal detector would be one which exhibits equal sensitivity for all the substances present, *e.g.*, by measuring some quan-

tity that is directly related to their weight. Such a detector does not exist, but the quartz crystal oscillation frequency detector[95] may be a promising beginning.

For a number of classes of lipids, *e.g.*, those which differ only in their degree of unsaturation, or by only one additional functional group present in triglycerides of fairly equal chain length distribution, the modified moving-wire detector may be a good approximation. This detector should give a signal that is proportional to the amount of carbon deposited on the wire. If it can be assumed that the compounds in a "lipid class peak" do not differ greatly in their relative carbon contents, the modified moving-wire detector[34] may be used to quantitate such peaks.

From our own work, however, it seems that there are also some class response differences that exceed those calculated from the relative carbon contents, and that peak sizes depend on the nature of the eluting solvent.

The quantitation of peaks that represent individual substances is straightforward (*e.g.*, calibration by injection of a known amount, use of internal standards).

For detection with a moving-wire transport FID, we usually find a relative standard deviation of *ca.* 5% in the peak area[106,153], although this value may sometimes be exceeded if flow variations occur during gradient elution.

For UV detection, the manufacturers usually claim 0.5–1% relative standard deviation for peak areas (replicate injections of one preparation), but the reproducibility should perhaps more realistically be put at a relative standard deviation of 2.5–3%[84,196]. This value may increase further if a non-UV-absorbing lipid must first be derivatized so as to permit UV detection.

A major drawback of quantitative LC work is the fact that most electronic integrators (which have been designed for GLC signals) are difficult to use with a liquid chromatograph, particularly in non-linear gradient elution.

6. CONCLUSION

Most of the papers reviewed here have been arranged in the form of a table (Table 1). This was thought to be the most useful way to provide the reader with easy access to information such as "which technique has been applied to particular types of samples?". Table 1 also includes a number of references that are not specifically mentioned elsewhere in the text[231–256]. Table 1 should primarily be an aid to the practising LC chemist.

The literature reviewed here shows that liquid chromatography should have great potential for applications to work with lipids, particularly for the analysis of glycerides, modified glycerides, polar lipids and high-molecular-weight or polar oleochemicals, but it is still handicapped by equipment problems for the analysis of these non-UV-absorbing classes of substances.

The present preponderance of publications dealing with steroids and vitamins (Table 1) does not seem to result from a particularly urgent need but rather from the comparative convenience of UV detection and the pharmaceutical market orientation of most of the LC equipment manufacturers.

The problem of detection has not yet been solved properly, either from a sensitivity point of view or from that of the reproducibility in quantitation, and gradient elution systems for more than two solvents are just beginning to appear on the market. Further improvements in these areas are to be expected soon.

Some of the traditional lipid separation techniques are difficult to utilize in HPLC, *e.g.*, the solvent selectivities[6] for lack of solvent switching facilities, and some of the sorbent selectivities for lack of suitable hard-core column filling materials for high-pressure LC. As an example, there is no high-pressure counterpart for Sephadex, and such selective effects as preferential hydroperoxide sorption, ketone exclusion and aromatic adsorption, mechanisms which are very different from those which occur on silica gel surfaces, cannot be carried out with present-day HPLC column filling materials.

7. ACKNOWLEDGEMENT

The author is indebted to Miss K. Maack for her help in preparing the manuscript.

8. SUMMARY

Equipment and techniques used for the LC of lipids are discussed. Applications to biological lipids, food lipids and technical lipids and oleochemicals are reviewed.

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