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Review

On-line coupled liquid chromatography–gas chromatography

Tuulia Hyötyläinen*, Marja-Liisa Riekkola

Laboratory of Analytical Chemistry, Department of Chemistry, University of Helsinki, P.O. Box 55, FIN-00014 Helsinki, Finland

Abstract

On-line coupled liquid chromatography–gas chromatography (LC–GC) is a powerful technique that combines the best features of LC and GC and is ideal for the analysis of complex samples. This review describes the unique features of on-line coupled LC–GC. The different interfaces and evaporation techniques are presented, along with their advantages and disadvantages. Guidelines are given for selecting a suitable LC–GC technique and representative applications are noted.
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Keywords: Reviews; Liquid chromatography–gas chromatography; Interfaces, LC–GC; Evaporation methods

Contents

1. Introduction 358
2. Reasons for selecting on-line LC–GC 358
3. Apparatus and conditions for on-line coupled LC–GC 359
   3.1. Liquid chromatography in LC–GC 360
   3.2. Interfaces and evaporation techniques in LC–GC 362
      3.2.1. On-column interface with retention gap techniques and concurrent solvent evaporation 363
         3.2.1.1. Retention gap techniques 363
         3.2.1.2. Concurrent solvent evaporation 364
      3.2.2. Loop-type interface with concurrent solvent evaporation 364
      3.2.3. Vaporiser interface 364
      3.2.4. Removal of solvent vapours 365
   3.3. Special solutions for coupling of RPLC to GC 366
      3.3.1. Direct transfer of RPLC eluents 366
      3.3.2. Indirect methods 367
4. Choice of interface and evaporation method 368
5. Setting up an LC–GC method 370
6. Selected applications 371
   6.1. Food samples 371
   6.2. Petrochemical and industrial samples 376
   6.3. Environmental applications 377
   6.4. Pharmaceutical and biological samples 378
   6.5. Comprehensive LC×GC 379

*Corresponding author. Tel.: +358-919-150-267; fax: +358-919-150-253.
E-mail address: tuulia.hyotylainen@helsinki.fi (T. Hyötyläinen).

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6.6. Coupling an extraction system on-line to LC–GC 379
7. Concluding remarks 380
References 381

1. Introduction

Multidimensional chromatographic techniques, such as on-line coupled liquid chromatography–gas chromatography (LC–GC), are excellent tools for the analysis of complex samples. Coupling LC with GC allows combination of the best features of both. The high sample capacity and wide range of separation mechanisms of LC can be utilised in selective clean-up, fractionation and preconcentration of the sample. For the final separation, GC offers high separation efficiency and a variety of selective detection methods. There are many benefits in combining the two techniques: the analysis is faster, less solvent is needed and the cost of analysis decreases. The analysis and sample preparation take place in a closed, usually automated system, where the risks of sample loss and contamination are minimised and, thus, the reliability and repeatability of the analysis are improved. In addition, the negative effects of atmospheric oxygen and moisture are eliminated. One of the main benefits of LC–GC is that, because of the efficient clean up by LC, the whole sample fraction containing the analytes can be transferred to the GC. Since none of the sample material is wasted and the disturbing compounds are eliminated, sensitivity is high.

In contrast to conventional GC, the LC fractions transferred to the GC are typically as large as several hundred microlitres. This cannot be done without special interfaces. In addition, the LC eluent must be suitable for both LC and GC analysis. At present, most liquid chromatographic analyses are made in reversed-phase mode (RP). Most LC–GC methods, however, are normal-phase (NP) LC–GC. In part, this is because the organic eluents used in NPLC are typically compatible with GC, making the coupling simpler. Another reason is that many of the samples analysed by GC require extraction into organic solvent before analysis, and normal-phase separation is the obvious choice. If the whole range of analytical possibilities is to be exploited RPLC–GC must be used as well. The coupling of RPLC to GC demands skill and special techniques, however, since aqueous RPLC eluents are unsuitable for direct transfer to GC.

2. Reasons for selecting on-line LC–GC

The on-line LC–GC systems are understandably more complicated than single chromatographic methods. It would be unreasonable, therefore, to use LC–GC for simple analytical problems that are easily solved with traditional methods. Rather, LC–GC is appropriate for samples that are difficult or even impossible to analyse by a single technique. Off-line LC–GC techniques provide a good alternative to conventional techniques, when the sample amount is sufficient and the sensitivity required is not very high. They offer most of the benefits of on-line techniques and the instrumentation is more flexible. The sensitivity is, however, usually lower than in on-line methods because only a part of the sample is injected to the GC. Certainly, the sensitivity can be increased substantially through the use of off-line large volume injection. Off-line techniques have been widely applied in pesticide residue analysis, for example. The on-line technique is always the best choice, however, when large series of samples have to be analysed, the amount of sample is limited as, for example, in human exposure studies, or very high sensitivity is required.

Fig. 1 gives guidelines for choosing a LC–GC method. The main factors to consider in the selection are the complexity of the sample, i.e., the amount of matrix components, the characteristics of the analytes and the selectivity and sensitivity required. The analytes of interest should also be suitable for the final GC analysis, i.e., they should be sufficiently volatile and non-polar or derivatisation should be possible either before the analysis or on-line. In

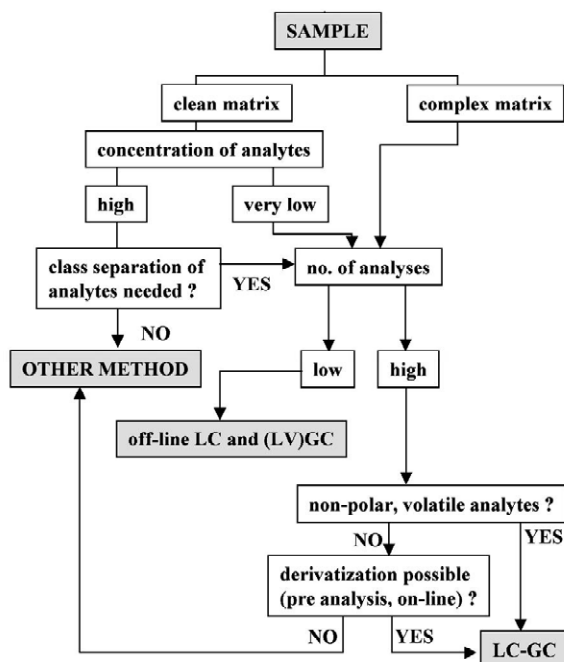

Fig. 1. When to use LC–GC: a decision tree.

addition, the number of samples to be analysed is relevant. If the number is small, there is usually no need for an automated method and the time-consuming development of such a method, and conventional methods will suffice. The more complex the sample matrix is, the more efficient the sample clean-up must be and then LC–GC is suited for the task. LC–GC may also be preferable for relatively clean samples if very high sensitivity or selectivity is required for the analysis for example, if the analytes of interest are present at trace level or group-type separation of the analytes is needed before the final analysis.

3. Apparatus and conditions for on-line coupled LC–GC

Fig. 2 shows a typical LC–GC instrument, which consists of a basic LC system, an interface valve and LC–GC interface, and a GC system with solvent vapour exit (SVE). One or two pumps are used in LC and often the separation is monitored with a UV detector. A detailed description of the instrumentation can be found in Ref. [1]. A number of interfaces have been developed for the LC–GC coupling, but early versions [2–6] have mostly been abandoned and only on-column [6–36], loop-type [37–65] and vaporiser interfaces [66–76] are commonly employed today. The GC part is typically a normal GC equipped with a suitable interface and a solvent vapour exit. When a vaporiser interface is used, the SVE is not always necessary.

In the development of an LC–GC method, the LC method is chosen first, keeping in mind the conditions required for transfer and GC analysis. The

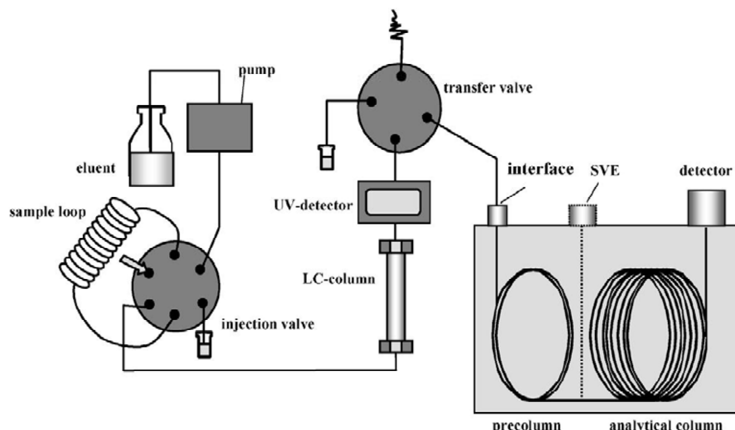

Fig. 2. Basic instrumentation for on-line coupled LC–GC.

Table 1
An approximate specification of volatility for LC–GC (and large volume GC)

Definition	Elution temp. (°C)	Example (alkane)
Volatile	≤120	C_6–C_{12}
Intermediate volatile	~120–170	C_{13}–C_{16}
Non-volatile	~170–300	≥C_{17}

selection of interface and evaporation technique is largely dependent on the volatility of the analytes. In GC, the dimensions of the retention gap and conditions during the transfer must be optimised for the selected interface and evaporation technique. The other GC conditions (i.e., column type, temperature program and detection) can then be selected quite independently. The volatility of analytes is defined in a comparison of their elution temperatures in GC (Table 1).

3.1. Liquid chromatography in LC–GC

The role of LC in the LC–GC coupling is to perform selective clean-up, concentration and/or fractionation of the sample. LC provides far better separation efficiency and selectivity than conventional sample preparation techniques. High efficiency columns can be used, and the facility to monitor the separation with the LC detector allows the conditions to be optimised quickly and precisely. In addition, the fraction(s) containing the analytes of interest can be accurately cut and transferred to the GC. Often it is not necessary, or even beneficial, to utilise the whole separation efficiency of the LC. The goal is simply to separate the analytes of interest from the matrix compounds.

In the development of an LC–GC method, the LC mode will be selected first, though the optimal conditions for GC analysis will need to be kept in mind in the design and optimisation of the LC conditions. The sample matrix and analytes of interest largely determine the LC mode. Table 2 presents some guidelines for the selection of a suitable LC method (RPLC methods not included). NPLC and size exclusion chromatography (SEC) are more easily coupled with GC than is RPLC, and should be used where possible. Many of the samples to be analysed by LC–GC will be extracted into organic solvent, and NPLC or SEC is the obvious choice therefore. SEC is suitable when a sample matrix contains high-molecular mass components, which are easily separated from small analyte molecules. The separation of compounds of similar molecular mass is poor, however. Furthermore, because SEC columns are of limited sample capacity, rather large columns have to be used in SEC and this in turn means that sample fractions are also large. A good example of an appropriate use of SEC–GC is the determination of pesticide residues in fatty matrices [26,30,52]. RPLC methods can be chosen when the sample is aqueous. However, extraction of analytes from the aqueous phase into suitable organic solvent and application of NPLC–GC instead will often be an easier solution than the RPLC–GC application.

The main parameters in LC are the column dimensions, the eluent and its flow-rate and the volume of the fraction containing the analytes (Table 3). Also, the interface and the evaporation technique used in GC are of importance in selecting the LC conditions. With the loop-type interface, the flow-rate of the LC can be chosen freely, but with other interfaces the LC flow-rate is limited by the evaporation rate of the solvent. Flow-rates are in the range of 100–500 μl/min in most LC–GC applications. Higher flow-rates can be used with the loop-type interface in which the LC flow-rate can be adjusted independently of the GC. The use of gradient elution is rather complicated and not advisable with most of the interfaces.

The internal diameter of the LC column is of importance because the smaller the diameter, the smaller is the volume of a peak. Likewise, the smaller the diameter, the lower is the flow-rate. When the I.D. of the column is decreased from 4.6 to 2 mm, the typical volume of LC fraction decreases from some 1000 to 200 μl, and the flow-rate decreases in the same ratio [1]. With capillary columns, the fraction volumes are only a few microlitres. However, also the sample capacity decreases with decreasing column I.D. and also the sensitivity of the whole analysis decreases. The capacity of the LC column is of importance when the analytes are present at trace levels in a matrix containing large amounts of disturbing components, which can overload the column. In the analysis of trace components in fatty matrices, for example, the

Table 2
Selection of LC method for LC–GC (RPLC methods are included)

Matrix	MW<1500–2000			MW<1500–2000					
LC method	SEC (GPC)			NPLC					
Analyte	Non-polar	(Intermediate) polar		Non-polar			(Intermediate) polar		
LC column	Styrenedivinylbenzene			Cyano, amino			Silica, alumina, cyano		
Analyte	Volatile	Intermediate	Non-volatile	Volatile	Intermediate	Non-volatile	Volatile	Intermediate	Non-volatile
Solvent	Cyclopentane/MeAc	THF/*n*-decane	THF	Isopentane, pentane, DEE	Hexane, heptane	Heptane, cyclohexane	Pentane/MTBE, pentane/DEE CH_2Cl_2	Hexane/MTBE, hexane/CH_2Cl_2 cyclohexane	Hexane or heptane/MTBE, hexane/CH_2Cl_2

DEE, diethyether; MeAc, methyl acetate; MTBE, methyl-*tert*.-butylether; and THF, tetrahydrofuran,

Table 3
The main factors of LC conditions for a selected LC column

Parameter	Comments	Typically
Column I.D.	*Effects on:* Volume of the transferred fraction: the smaller the better Sample capacity	2 mm
Eluent b.p.	Should be volatile, especially for volatile analytes	Hexane, pentane, DEE, MTBE, heptane or a mixture of these
Eluent flow-rate	Maximum rate determined by the interface and evaporation technique	0.1–0.5 ml/min
Gradient	Changes in eluent composition affect also the b.p. of eluent which can be problematic in on-column interface with PCSE	Not used often
Eluent modifiers	Non-volatile additives cannot be used	–

maximum tolerable amount of matrix components is about 1 mg for a silica column with I.D. of 2 mm [1]. A good compromise between tolerable fraction volume and sufficient capacity is a column with I.D. of 2 mm. Capillary columns can be used when the concentrations of the analytes are high relative to the matrix components. The column length is typically smaller than in conventional LC. Sufficient separation can often be achieved with columns 2–5 cm long.

In the case of NPLC–GC coupling, the choice of LC column and eluent is relatively straightforward. Silica, cyano or amino columns are used in most NPLC–GC applications. The eluent is typically a nonpolar, volatile solvent such as pentane or hexane or a solvent mixture of dichloromethane, methyl-*tert*.-butyl ether (MTBE), diethyl ether, isopropanol or acetonitrile with hexane or pentane. For the analysis of volatile compounds, the eluent should be volatile.

Size-exclusion chromatography (SEC) has also been used in LC–GC coupling [26,30,52] and, as in NPLC, the SEC eluent can be chosen so as to be suitable for the GC stage. To avoid possible LC interactions, however, the eluent should be sufficiently polar. In addition, for on-line SEC–GC the SEC step should be miniaturised to reduce the transfer volume. In practice, maximum I.D. of the column is 4.6 mm. The choice of eluent depends on the solubility of the sample and the volatility of the analytes of interest.

The coupling of RPLC with GC is demanding and this combination it is not widely applied. The coupling in RPLC–GC can be indirect or direct, as will be explained in more detail in Section 3.3. In indirect coupling the aqueous eluent is changed on-line to an organic solvent by solid-phase extraction, on-line liquid–liquid extraction or open tubular trapping. The LC conditions can be chosen relatively independently of the GC and, in many cases, buffer salts and gradient elution can be utilised. In direct coupling of RPLC–GC, on the other hand, the LC conditions must be selected with great care. Buffer salts cannot be used, and the flow-rate is limited by the slow evaporation rate of aqueous eluents. Most of the eluents are mixtures of water and methanol, and micro-LC has often been employed.

3.2. Interfaces and evaporation techniques in LC–GC

The volume of the fraction transferred from LC to GC is usually large and special techniques are needed for the transfer. An adequate separation of the analytes from the solvent is required and the analytes then have to be transferred to the GC column quantitatively. Several interfaces have been implemented for (NP)LC–GC [3–76]. On-column

[6–36], loop-type [37–65] and vaporiser interfaces [66–76] are almost exclusively used in LC–GC methods today. The on-column and loop-type interfaces are usually applied with retention gap techniques and concurrent solvent evaporation techniques, respectively, for the evaporation of the solvent (Table 4).

Interfaces can be grouped according to whether the solvent vapours are removed from the interface by overflow or by gas discharge [68]. Overflow occurs without gas flow; the vapours merely leave the column or chamber due to the expansion during evaporation, driven by vapour pressure higher than the pressure in the vapour outlet. There is no dilution with the carrier gas and the temperature of the evaporation site must therefore be at or above the pressure-corrected boiling point of the solvent. In gas discharge systems, vaporisation occurs in a stream of carrier gas. Solvent evaporation is possible below the boiling point of the solvent, owing to the dilution of vapours with the carrier gas. Gas discharge is more flexible than the overflow technique; temperature and gas flow can compensate each other and, unlike the overflow system, it can be used for partially concurrent evaporation. Overflow techniques, on the other hand, are simpler to optimise, because temperature is the only variable to be adjusted. Furthermore, the closure of the vapour exit is not critical in this system since the flow collapses at the end of the evaporation process.

3.2.1. On-column interface with retention gap techniques and concurrent solvent evaporation

The on-column interface is based on an on-column injector and gas discharge of the solvent vapours. The sample fraction is pushed to the retention gap by the LC pump in the flow of the carrier gas. The interface can be used with retention gap techniques or concurrent solvent evaporation. A long retention gap (5–10 m) is used with retention gap techniques and a short one (0.5–3 m) with concurrent evaporation. The internal diameter of the retention gap should be large (typically 0.53 mm I.D.), both to allow large enough capacity to retain the solvent and to obtain high gas flow through the precolumn and SVE for efficient evaporation [85].

Table 4
Characterisation of LC–GC interfaces and evaporation techniques

Interface	Evaporation
On-column	Conventional ret. gap
	Partially concurrent solvent evaporation
	Fully concurrent solvent evaporation
Loop-type	Fully concurrent solvent evaporation
	Fully concurrent solvent evaporation+co-solvent
Vaporiser	PTV split
	PTV splitless
	PTV overflow
	PTV solvent split
	Isothermal vaporisation

3.2.1.1. Retention gap techniques

In retention gap techniques, the fraction is transferred below the pressure-corrected boiling point of the solvent and the solvent is evaporated in the retention gap. The volatile analytes are reconcentrated by the solvent effects while the high boiling analytes are reconcentrated by the phase ratio focusing effect, which is based on the differences in the retention powers of the uncoated retention gap and coated separation column. It is essential in retention gap techniques that the retention gap is well wetted with the solvent. If the wettability is not sufficient, the film is not uniform, and the flooded zone increases in length and becomes unstable. The phase soaking effect improves the retention of the analytes eluting near the solvent due to the increased retention power of the stationary phase film.

An on-column interface used with retention gap techniques allows the analysis of both volatile and non-volatile analytes. The conventional retention gap techniques are well suited only for relatively small sample fractions, but by applying partially concurrent evaporation the volume of the sample fraction can be enlarged to some 1000 μl. The drawback of the techniques is that three interdependent factors have to be optimised: length of the flooded zone determining the length of the retention gap, the transfer rate and the rate of evaporation of the solvent [8,80–82,85]. If SVE is used, the closure of the exit is critical. Furthermore, since wettability of the retention gap is required for solvent trapping, direct transfer of aqueous solvents is limited. Other solvents difficult to work with are acetonitrile, benzene, dioxane, dichloromethane and toluene. The LC separation has to be efficient for retention gap

techniques to be used since non-volatile impurities tend to stick to the wall of the retention gap, altering the retaining power of the column.

3.2.1.2. Concurrent solvent evaporation

It is also possible to use the on-column interface with fully concurrent solvent evaporation. A short piece of retaining precolumn (0.5–3 m) is attached to the on-column interface [79]. The precolumn should be short to obtain high gas flow through the precolumn and SVE. High gas flow prevents back flow of solvent vapours to the injector and gas supply. The solvent vapours are diluted with carrier gas, and oven temperatures can be lower, improving the retention of volatile analytes.

3.2.2. Loop-type interface with concurrent solvent evaporation

The loop-type interface has been specially designed for LC–GC coupling. The sample fraction is collected in a loop in a multiport valve, from which the carrier gas pushes it to the uncoated GC column. The interface is usually used with the fully concurrent solvent evaporation technique (FCSE), where the solvent is concurrently evaporated during the transfer. The oven temperature is kept above the boiling point of the solvent at the applied inlet pressure. The vapour pressure of the evaporating solvent quickly exceeds the inlet pressure and prevents further entry of the plug of liquid into the retention gap. The technique is a vapour overflow technique, which means that vapours leave the column by expansion and the carrier gas flow starts only after the evaporation is complete.

During the transfer, almost all of the solvent is evaporated concurrently and virtually no liquid floods to the GC column. Thus, there is no solvent trapping and volatile components are lost by co-evaporation with the solvent when SVE is used. If desired, solvent trapping can be achieved in loop-type interface by adding a small amount of higher boiling co-solvent to the eluent (FCSE with co-solvent trapping), which provides the solvent trapping conditions for the volatile components [1,30,108]. To simplify the optimisation, the co-solvent is usually chosen such that it forms an azeotropic mixture with the main solvent and the mixture evaporates in known ratio of the components virtually independent of the pressure.

The advantage of the loop-type interface is that the operation is simple. The transfer is almost completely self-regulated; the only parameter to be adjusted is the oven temperature, and only a short uncoated precolumn is needed. Large sample volumes up to several millilitres are easily transferred. At least in theory, the method is suitable for aqueous eluents as well. The optimisation is somewhat more complicated if co-solvent trapping is applied: the co-solvent and its concentration need to be considered together with the oven temperature. The loop-type interface with FCSE is only suitable for relatively non-volatile analytes, however. Without co-solvent trapping, the first sharp peaks can be expected only some 60–100 °C above the transfer temperature, varying with the eluent and the sample volume [1,38].

3.2.3. Vaporiser interface

The third interface widely used in LC–GC coupling is the vaporiser interface [66–93]. Most applications involving the vaporiser interface make use of a programmable temperature vaporiser (PTV) or a hot vaporising chamber. The vaporising chamber is usually packed with some inert material (glass wool, glass beads, Carbofrit) or an adsorbent such as Tenax [86,89]. The in-line vaporiser does not have a separate chamber: a piece of wire or end-sealed fused-silica capillary is installed inside the precolumn and the whole system is fitted in a heating system [71].

There are several ways to perform the transfer: PTV solvent split, PTV large volume splitless transfer, PTV vapour overflow transfer with or without splitting, and various modifications of these techniques [66,73,72,74,90,92] (see Table 5). In PTV solvent split transfer, there is no need for SVE.

In large volume transfer using PTV solvent split mode small volumes can be transferred to the liner at high flow-rate, the maximum flow being determined by the volume of the liner. As an example of this the maximum volume for a packed liner with I.D. of 4 mm is 100–150 μl [90]. Large volumes have to be introduced using a flow-rate that is close to the rate of solvent elimination. The solvent–analyte separation and, thus, the losses of volatile analytes can be minimised by using a liner packed with appropriate

Table 5
Characterisation of the vaporiser interfaces for LC–GC

Technique	Temperature during transfer	Max fraction volume (μl)	Volatility application range[a]
PTV split	Under solvent bp	1500	≥C13, C10–C30 (Tenax)
PTV splitless	Under solvent bp	50–100	≥C10
PTV vapour overflow	Over solvent bp	1500	≥C16
PTV solvent split	Under solvent bp	10–500	≥C14
Isothermal hot vaporisation	Over solvent bp	1500	
Split			≥C16
Splitless			≥C14
Splitless overflow			$\geq C_{18}$
Precolumn solvent split			≥C16

[a] For typical organic solvents.

material. In PTV large volume splitless transfer, the solvent vapours are vented via the GC column or the retaining precolumn and SVE, and the volatile analytes are trapped in the stationary phase of the column. The disadvantage of this technique relative to the PTV split technique is long evaporation time. The PTV vapour overflow differs from the two techniques described above in one major point: there is no gas flow during the introduction of the sample. Solvent vapours are removed through the purge exit as the vapours expand. It is also possible to use the PTV interface as an isothermal vaporisation chamber. The chamber is kept well above the boiling point of the solvent. Vapours are discharged through a coated precolumn and an early vapour exit located between the precolumn and the analytical column. The solvent–analyte separation takes place in the precolumn instead of the vaporising chamber. The PTV solvent split technique is suitable for medium and less volatile compounds, but not for thermolabile compounds. The PTV large volume splitless transfer is also suitable for relatively volatile compounds, while the over flow technique is appropriate only for less volatile compounds.

3.2.4. Removal of solvent vapours

The large fraction volume transferred from LC to GC means that the amount of solvent vapours is very large. If the solvent vapours are pushed through the whole analytical column, they slow down the evaporation process and the time required for the removal of solvent vapours is excessive. Both to increase the evaporation rate and to protect the GC detector from the large solvent vapour cloud, it is advisable to use a remove the vapours before the analytical column. The solvent vapour exit is usually applied with on-column and loop-type interfaces, placed between the GC precolumn and the analytical column. With vaporiser interfaces utilising the split valve, the SVE is not necessary.

Timing of the closure of the SVE is critical in retention gap techniques since the volatile analytes trapped by the solvent film become highly mobile at the end of the evaporation and are quickly lost through the exit if the closure occurs too late. Accurate closure of the split exit valve in vaporiser interfaces corresponds to the closure of the SVE in on-column and loop-type interfaces and it is equally critical. In SVE, a short piece of retaining precolumn has usually been attached between the retention gap and analytical column, but recent studies show that this is not necessary [80–82]. More simply, a restrictor capillary can be placed in the vapour outlet, or a short smaller I.D. retention gap can be introduced before the SVE. A restrictor reduces the gas flow-rate avoiding plug formation and strong pressure drop, which could cause the solvent also to evaporate partially from the rear of the flooded zone so disturbing the solvent trapping process [80–82].

At present, the timing of the closure of the exit is usually determined empirically, by checking for losses of volatile analytes or by applying the flame method. If solvent trapping is applied in retention gap techniques, the vapour exit should be closed shortly before or after the completion of solvent evaporation. The closure is especially critical if high evaporation rates are utilised. An automated method for the accurate closure is preferable, since even a

minor change in conditions between runs may change the optimum time of closure by a few critical seconds. Accurate timing of the closure of the SVE can be achieved by measuring the temperature changes during the evaporation from the outer wall of the precolumn [83] or by measuring the changes in the carrier gas flow-rate during the evaporation process when a pressure-regulated system is used [84]. The measurement of temperature change is based on the simple physical phenomenon of cooling of the evaporation area (that is, the capillary surface) as the liquid evaporates. The measurement of change in the gas flow-rate is based on the decrease in the flow-rate during the evaporation as the solvent vapours partly substitute for the carrier gas. The gas flow-rate increases again after the evaporation is complete [84]. However, since the volatile analytes are released together with the last portion of solvent, closure based on the measurement of gas flow-rate will always occur slightly too late. Furthermore, admixture of solvent vapours with a viscosity much lower than that of the carrier gas (particularly true for helium) could result in substantial increase of the total flow-rate; that is, under certain conditions the flow-rate of the carrier gas will not change at all [79].

3.3. Special solutions for coupling of RPLC to GC

As mentioned earlier, RPLC–GC coupling is much more demanding than coupling of NPLC with GC and the interfacing techniques used in NPLC–GC generally do not work well for RPLC–GC.

Water is a not a good solvent for GC, primarily because it hydrolyses the siloxane bonds in GC columns causing deterioration of the column performance. Furthermore, wettability of retention gaps is essentially not achieved irrespective of the type of deactivation applied to the capillary surface. The evaporation rate of water as of methanol and acetonitrile is low, making the transfer of large sample volumes time-consuming. In addition, water and water-containing eluents are very poor solvents for those useful solvent effects such as solvent trapping and phase soaking.

As illustrated in Fig. 3, there are two ways to solve the problems related to RPLC–GC coupling. Direct solutions to the problem of aqueous eluents rely on special techniques, whereas indirect solutions avoid them by phase switching, i.e., replacing the water with suitable organic solvent before introduction to GC. The solutions for RPLC–GC have been reviewed in detail [161].

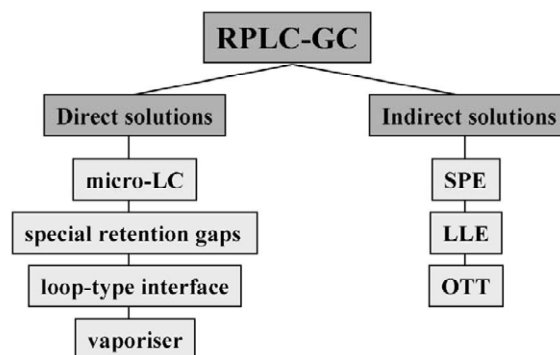

Fig. 3. Different solutions for the coupling of RPLC and GC.

3.3.1. Direct transfer of RPLC eluents

Direct transfer from RPLC to GC is possible if the RPLC eluent does not contain non-volatile additives, such as buffer salts. The main solutions developed for direct transfer are micro-LC–GC coupling, which allows the direct transfer of aqueous eluents [39,94–100], retention gap techniques using special deactivated retention gaps, the loop-type interface and the vaporiser interface. Typically, the eluents used with direct transfer techniques are water–methanol or water–acetonitrile.

In micro-RPLC the sample fractions transferred from RPLC to GC are small (usually less than 10 μl) and the low evaporation rate of water is not a problem. The coupling is relatively easy therefore. The disadvantage is the low sample capacity of the LC column which makes the sensitivity of the LC–GC method rather low.

Retention gap techniques reported for RPLC–GC utilise retention gaps coated with Carbowax-type phases, aminopropyltriethyl-siloxane and OV-1701-OH [17,96,101–104]. None of the phases has been proven of practical use in RPLC–GC. Although some applications have been claimed to be successful [17,101,104], failure of these retention gaps to withstand water in the long run has also been reported [105–107].

No wettability is required in loop-type interface and aqueous eluents can be transferred directly to GC. The deteriorating effect of water vapour is far less pronounced than that of liquid water. However, in the absence of solvent and phase soaking effects, it is practically impossible to analyse volatile analytes. Only analytes with elution temperatures above 230–260 °C can be analysed [108]. Co-solvent trapping allows the analysis of analytes eluting above 110–140 °C, as has been described with butoxyethanol as co-solvent [109,110].

Several studies have been published on use of the vaporiser interface for direct transfer of aqueous effluents [68,110–126]. With direct transfer of water to the vaporising chamber, it is not possible to use sub-zero initial conditions nor to create a solvent film in the porous glass bed inside the liner, these being the typical ways to reduce losses of volatiles in PTV techniques. To trap volatiles, liners have to be packed with an adsorptive materials, such as Tenax, which in turn prevents the analysis of high boiling compounds. In principle, there are two ways to separate water from the analytes in the liner of PTV: by evaporation or by non-evaporative mode. The PTV interface with evaporative mode can be used only for relatively small fraction volumes owing to the slow evaporation rate of water. It should also be noted that the breakthrough volumes for packed PTV liners with water as solvent may be very small [73,87].

The vaporiser-precolumn solvent split-gas discharge interface differs slightly from the conventional PTV interface. In this system, the vaporising chamber is connected to a short coated precolumn and further to the SVE and the analytical column [86,114,115]. The drawback of the vaporiser interface is that the methods are limited to relatively non-volatile analytes [75] unless the liner is packed with an adsorbent material. However, if the liner is packed with adsorbent, desorption of high boiling compounds is difficult. To overcome this problem, a new version of the vaporiser interface has been developed, in which there are two PTVs and two carrier gas inlets, as shown in Fig. 4 [124]. The first PTV is used at constant high temperature to vaporise the sample. It is connected to a short piece of retaining precolumn, which is attached to the second PTV and the analytical column via a T-piece. The second PTV is packed with trapping material and is located just before the solvent vapour exit. The idea is relatively simple: after the sample has been vaporised, the high boiling components are trapped in the stationary phase of the retaining precolumn, while the volatile analytes are trapped in the trapping material of the second PTV. After the transfer and solvent evaporation, the carrier gas flow is switched so that the second PTV is used as the inlet and, with increase in the PTV temperature the analytes trapped in the second PTV are released and the analysis commences.

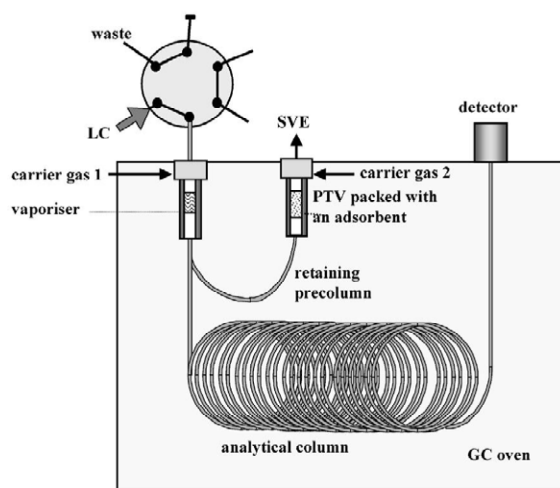

Fig. 4. RPLC–GC coupling using dual PTV system.

Several RPLC–GC applications have been published which rely on PTV packed with Tenax, and operate at low temperatures (10–30 °C) and with very high LC flow-rates (<1 ml/min) [111–113,116–120,122,125,126]. However, since it is not possible to evaporate aqueous eluent under these conditions, so the system is actually based on (partial) solid-phase extraction [79]. A similar set up is used in a modified PTV interface, but the temperature is higher (80 °C) and the flow-rate of the aqueous eluent is considerably lower (0.1 ml/min) [123,127].

3.3.2. Indirect methods

The three major indirect methods for coupling RPLC with GC, in which water is replaced with suitable organic solvent by phase-switching, are

solid-phase extraction, on-line liquid–liquid extraction and open tubular trapping [128–160]. The problems with water in GC are thus eliminated. In addition, the LC conditions can be chosen much more freely than with direct RPLC–GC methods. It is also possible to add an on-line derivatisation step between the LC and GC after water has been eliminated [143–147].

One useful way to eliminate water before GC analysis is by solid-phase extraction (SPE). The analytes are trapped in a short column packed with suitable stationary phase (usually C_{18} material). The trap can be placed after the LC column [128,129]. Also, several SPE–GC methods, which resemble RPLC–GC, have been developed [130–142]. After trapping, and before elution of the analytes, the SPE column is often dried with a gas flow. Another possibility is to install a special drying column, packed with copper sulphate or silica to remove water, after the SPE column [136]. The elution is done with a solvent suitable for GC analysis, often ethyl acetate or *n*-propanol. SPE with thermal desorption (SPETD) is similar to normal SPE methods, with the difference that the analytes are eluted from the stationary phase by heating the trap [137,138]. The trap is often located directly at the GC injector. The carrier gas supply can be adjusted so that there is a counterflow from the GC column to the injector, preventing water from entering the GC column during sampling and drying. Since many commonly used solid-phase materials cannot withstand elevated temperatures, different packing materials are used in SPETD and SPE. Tenax is one of the most common packing materials for SPETD, since it has sufficient retention power for analytes, good thermal stability and poor enough interaction with water to allow optimal drying. The drawback of SPETD is that many analytes are too efficiently trapped on the stationary phase and do not desorb upon heating.

The phase switching can also be done with continuous liquid–liquid extraction (LLE) [44,141–156]. In this technique, the aqueous eluent is mixed with organic solvent in a T-piece, and the analytes are extracted into the organic solvent in an extraction coil. After the extraction, aqueous and organic phases are separated in a phase separator, which can be a simple T-piece, membrane separator or sandwich-type separator [44,143–156]. The distribution ratio can be maximised through a careful choice of organic solvent, pH of the aqueous phase and ratio of the two phases. Another straightforward way to enhance the extraction ratio is to raise the temperature [146,147].

The open tubular trap consists of a capillary column with a thick stationary phase (typically about 5–100 μm) of the same material as in the GC columns [157–160]. Often the trap is located in a separate GC oven. The effluent is slowly flushed through the column, which is then dried with gas flow, and finally the analytes are either eluted with solvent or desorbed by heating the column. The extraction efficiency can be enhanced by coiling or bending the extraction tubing, which enhances the tubular flow in the tube and so increases the contact of the analytes with the stationary phase [159]. Desorption with solvent (OTT) is more efficient than thermodesorption (OTTTD), but with OTT the stationary phases must be well cross-linked. The advantage of OTT over SPE and SPETD is that the elimination of water is much more efficient. In addition, the stationary phases are thermally stable and inert and have well-known retention characteristics. The main disadvantage is that the trapping of the analytes to the stationary phase of the column is not as efficient as in SPE methods.

4. Choice of interface and evaporation method

The choice of the interface and the evaporation technique mainly depends on the application. The advantages and disadvantages of the different techniques are listed in Table 6. The main parameters to be considered are the volatility of the analytes and the sample volume. For RPLC–GC, the direct approaches relying on vaporiser interfaces appear to be the most promising. Among the indirect approaches (RPLC)–SPE–GC has given good results.

The efficiency of solvent–analyte separation during evaporation of the solvent determines the suitability of the interface for the analysis of volatile analytes. Volatile analytes are totally lost if they evaporate and leave the GC system together with the solvent vapours. The most important factor affecting the solvent–analyte separation is the retention power in the interface. In retention gap techniques, the

Table 6
Comparison of different evaporation techniques

Systems	Requirements for LC	Max/min fraction volume (μl)	Elution T of first sharp peaks (°C)[a]	Optimisation of parameters	Suitability for aqueous fractions	Advantages	Disadvantages
On-column with conventional ret. gap technique	Restricted LC flow-rate, difficult to use gradient elution	10–250	Below 100	Relatively difficult	Poor	Suitable for volatiles[b]	Limited sample volume, requires long retention gap.
On-column with PCSE	Restricted LC flow-rate, difficult to use gradient	50–1000	Below 100	Difficult	Poor	Suitable for volatiles[b]	Optimisation demanding
On-column with FCSE	Restricted LC flow-rate, gradient elution possible	50–1500	Above 120–140	Relatively easy	Poor	Suitable for large volumes, only a short ret. gap is required	Not suitable for volatiles[b]
Loop-type with FCSE	LC flow-rate can be chosen freely, possibility to use gradient elution	20–20 000	Above 120–140	Easy	Relatively good	Only transfer temperature has to be optimised, suitable for large volumes	Not suitable for volatiles[b], loop size restricts the fraction size
Loop-type with FCSE+ co-solvent	LC flow-rate can be chosen freely, co-solvent must suit also LC	20–20 000	Below 120	Relatively easy	Relatively good	Suitable for large volumes and also for relatively volatile analytes	Loop size restricts the fraction size, the co-solvent must be chosen carefully
Vaporiser	Restricted LC flow-rate, possibility to use gradient elution	Up to millilitres	Depends on the technique[c]	Relatively difficult	Good	Tolerates dirty fractions, suitable for large volumes	Optimisation demanding

[a] For organic solvents typically used in GC and NPLC–GC.
[b] Volatility range from C_6 to C_{14}.
[c] See Table 5.

flooded zone acts as the stationary phase; in loop-type interface and in vaporiser-precolumn solvent split systems, it is the retaining precolumn, and in other vaporiser techniques it is the packing material of the vaporising chamber.

The separation of solvent and analytes during the evaporation is most effective in on-column interface utilising retention gap techniques, and this combination is the best option for volatile analytes. In the optimum case, the volatiles are totally trapped in the flooded solvent zone and are released with the final portion of the evaporating solvent. The loop-type interface with FCSE is not at all suitable for highly volatile compounds. Even when very volatile eluent (pentane, diethylether) is used, sharp peaks can be obtained only for analytes eluting above 110–140 °C [1,38]. The vaporiser interface with solvent split and the vaporiser with liner packed with adsorbent are suitable for moderately volatile analytes ($\geq C_{10}$) when the conditions are carefully optimised.

There are several different, often interdependent parameters that have to be optimised for the transfer of the fraction of interest from LC to GC. The parameters of the interfaces based on gas discharge (on-column with retention gap techniques, most vaporiser interfaces) tend to be more difficult to optimise than interfaces based on vapour overflow (loop-type interface, vaporiser with vapour overflow). Usually the optimisation is done by trial and error.

The oven temperature during transfer is perhaps the most critical parameter. A difference of just a few degrees may well determine whether the separation succeeds. Since most large volume transfer techniques include solvent venting (SVE or split exit), this gives one more parameter to be adjusted. Empirical procedures use peak shapes and losses of analyte material as criteria for optimal conditions. Alternatively, the evaporation rate can be measured and adjusted with the help of the flame method. It is also possible to use programs for extrapolating the required conditions from a set of previously determined evaporation rates. The optimal oven temperature for the transfer is relatively easy to determine in overflow techniques, since the boiling point of the solvent is directly dependent on the inlet pressure and the boiling point can be calculated. The situation is more complicated in gas discharge systems because the carrier gas flow dilutes the eluent vapours, and thus the boiling point is decreased. In gas discharge methods, therefore, it is better to refer to the dew point of the solvent, below which the solvent vapours start to condense and above which there is no flooding liquid. With high carrier gas flow-rates, the dilution is greater and the dew point is lowered. However, the increase in gas flow-rate also requires an increase in the inlet pressure, which partially eliminates this benefit. As there is a pressure gradient inside a capillary column and, in addition, the gas flow-rate along the capillary is not constant during the transfer, it is difficult to calculate the dew point accurately. The dew point of a solvent is dependent on the boiling point of the solvent and the applied pressure. The dew point can be measured by using a thermocouple attached to the outer wall of the column in similar manner to measurement of the accurate closure time of the SVE [83].

5. Setting up an LC–GC method

It is difficult to give exact guidelines for setting up an on-line LC–GC method. Tables 2, 5 and 6 can be used in the selection. The LC mode is selected according to the sample, as described earlier. The volatility of the analytes is the main factor to be considered in choosing the interface and evaporation technique (Tables 5 and 6), and these, in turn, determine the appropriate LC eluent and column (Table 2). Often, compromises will have to be made in the LC conditions in order to meet the requirements for the optimal interface and evaporation. NPLC–GC with a volatile eluent or eluent mixture should be chosen, if possible. The on-column interface with partially concurrent eluent evaporation is suitable for most (NP)LC–GC applications.

The optimisation of the LC–GC conditions is dependent on the interface and transfer technique chosen. In general, after the LC mode has been selected, the LC conditions (column, eluent) should be optimised such that sufficient separation efficiency is obtained while keeping the fraction volume sufficiently small (<800 μl). The optimal LC flow-rate is dependent on the interface. As was stated above, the flow-rate can be chosen freely with

the loop-type interface but with the on-column and vaporiser interfaces it must be carefully optimised according to the evaporation rate of the LC eluent. Guidelines for selecting the preliminary conditions can be found in the literature (see, e.g., Refs. [1,8,38]). In practice, however, fine-tuning of the conditions will usually be required. With volatile analytes, the timing of the closure of the SVE or the split exit in the vaporiser interface is critical and it should be optimised carefully.

The peak shapes in a GC chromatogram give valuable information for optimising a method or trouble-shooting an existing method. Fig. 5 shows how chromatograms obtained with improper transfer conditions lead to distorted peaks. In on-column transfer, for example, if the transfer flow-rate is too high or the oven temperature too low, the flooded zone will reach the coated column which will result in split peaks (Fig. 5A). A similar effect will be seen with the loop-type transfer if the oven temperature is too low during the transfer. Too high transfer temperature, too high rate of evaporation or too late closure of the exit (SVE or split exit in PTV), on the other hand, will result in losses of the volatile components (Fig. 5B). Too early closure of the exit is not advisable either, because the solvent peak then becomes very large (Fig. 5C).

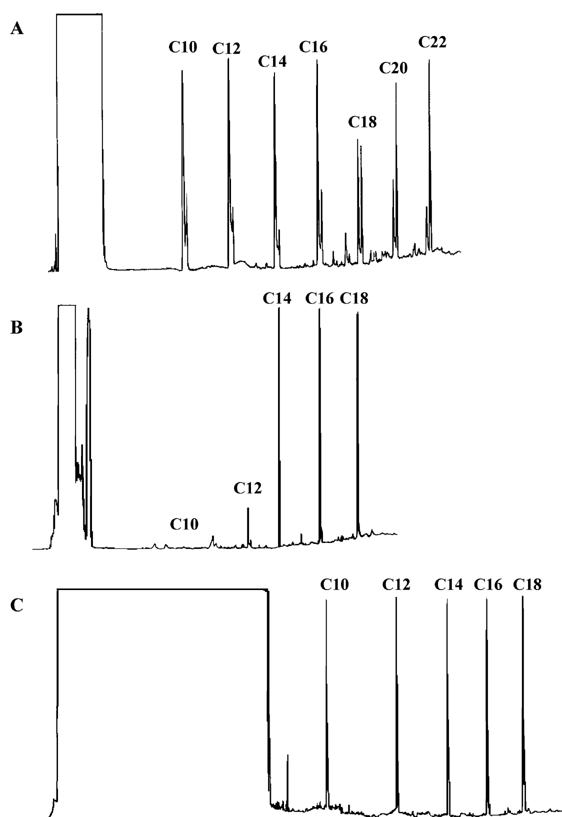

Fig. 5. Trouble-shooting: (A) split peaks owing to the too fast transfer flow-rate, (B) peaks for volatile analytes are too small owing to the too late closure of the SVE, and (C) the solvent peak is excessive owing to too early closure of SVE. Transfer conditions: on-column interface with PCSE, alkanes in *n*-heptane, oven temperature during transfer 80 °C, LC flow-rate in (A) 280 μl/min and (B,C) 200 μl/min.

6. Selected applications

LC–GC methods have been applied in the analysis of food, fossil fuel, agricultural, medical, environmental and other complex samples. Some interesting applications are described in the following, and recent applications (1997–2002) are summarised in Tables 7–10. Comprehensive reviews covering different types of LC–GC and related applications can be found in Refs. [69,79,161–175].

6.1. Food samples

On-line NPLC–GC is frequently used in routine analyses of food samples and several RPLC–GC applications have been developed as well. The LC–GC applications in food analysis include determination of raspberry ketone in raspberry sauce [37] and of mineral oil and packing material contaminants in foods [28,63,176,177], determination of the composition of edible oils [16,15,24,120,127], analysis of various compounds in edible oils and fats [9,32,47,50,51,56,58,64,111,113,117–119,126,176–183,187,188]; determination of the enantiomeric composition of edible oils [122,125,185], analysis of chiral lactones in fruit products [116], identification of irradiation of foods through the radiolysis products [8,22,23,29,127,179,180] and determination of pesticides in olive oils, edible oils, fats and red wines [30,55,114]; organochlorine compounds, PAHs and PCBs in edible oils and fats [25,45,182,186]; and oryzanol in rice bran oil [65].

The successful NPLC–GC analysis of the origin of olive oils demonstrates the very high separation

Table 7
Analysis of food samples by LC–GC

Sample	Method	LC conditions	Interface	Transfer conditions (temperature, carrier gas)	Refs.
γ-Oryzanol in rice lipids	NPLC–GC–FID	25×3 mm I.D., Nucleosil 50-5, hexane: isopropanol (98.5/1.5) at 0.45 ml/min	Loop-type with FCSE	60 °C, H_2 at 75 kPa	[65]
Steryl esters in cocoa butter	NPLC–GC–FID	150×2.1 mm Zorbax-SB (5 μm), CH_2Cl_2: ACN:*n*-hexane (2:0.1:97.9), 0.2 ml/min	Loop-type with FCSE	120 °C, He at 250 kPa	[64]
Volatiles in edible oil	RPLC–GC–FID	50×4.6 mm Kromasil-100-10C4, H_2O: MeOH (10:90, v/v), 1.8 ml/min	Modified PTV	PTV at 10 °C, He at 800 ml/min	[126]
Furan fatty acids in olive oil	NPLC–GC–PID	250×2 mm Lichrospher (5 μm), hexane: MTBE (97.8/1.2), 0.5 ml/min	On-column with PCSE	80 °C, H_2	[32]
Enantiomers of γ-lactones in edible oils	RPLC–GC–FID	50×4.6 mm Kromasil-100-10C4, H_2O: MeOH (10:90), 1.4 ml/min	Modified PTV	21 °C, He at 1800 ml/min	[125]
Organophosphorus pesticides in olive oil	GPC–GC–FPD	Methyl acetate/cyclopentane/nonane, 0.3 ml/min	On-column with PCSE	77 °C	[30]
Free sterols, tocopherols and squalane in edible oils	RPLC–GC–FID	50×4.6 mm Vydac 214 TBP10, H_2O: MeOH (22:78, v/v), 2 ml/min	Modified PTV	21 °C, He at 800 ml(min	[117,119]
Free erythodiol and uvaol in olive oil	RPLC–GC–FID	Vydac 214 TBP10, H_2O:MeOH (22:78, v/v), 2 ml/min	Modified PTV	PTV at 21 °C, He at 800 ml/min	[118]
Acylglycerols in vegetable oil	NPLC–GC–FID	*n*-hexane:ACN (98.5/1.5, v/v), 0.2 ml/min	Loop-type with FCSE	130 °C, H_2 at 80 kPa	[58]
Enantiomeric composition of filbertone in olive oil	RPLC–GC–FID	Vydac 214 TBP10, H_2O:MeOH (65:35, v/v), 2 ml/min	Modified PTV	PTV at 21 °C, He 1500 ml/min	[120,122]
Enantiomers of chiral lactones in foods	RPLC–GC–FID	Vydac 214 TBP10, H2O:MeOH (35:65, v/v), 1.8 ml/min	Modified PTV	PTV at 21 °C, He 1500 ml/min	[116]
Pesticides in red wine	RPLC–GC–FID	10×1 mm AsahiPak (5 μm), H_20:MeOH (20:80), 0.7 and 0.1 ml/min	Vaporiser interface	PTV at 300 °C, He at 120 kPa, GC oven at 60 °C, He through SVE 600 ml/min	[114]
Radiation-induced hydrocarbons in fish and prawns	NPLC–GC–FID	124×4 mm LiChrospher Si60 (5 μm), *n*-hexane, 0.2 ml/min	On-column with PCSE	79 °C, He at 0.9 bar	[29]
Organochloric compounds in milk fat	NPLC–GC–°CD	30×2.1 mm silica, *n*-hexane, 0.15 ml/min	On-column with PCSE	78 °C at 80 kPa	[25,195]
Mineral oil contamination in food	NPLC–GC–FID	10×1 Spherisorb Si5, *n*-hexane, 0.2 ml/min	On-column with PCSE	82 °C at 80 kPa	[28]
Paraffins in food stimulants and packaging materials	NPLC–GC–FID	25×4.6 mm LiChrospher SI60 (5 μm), *n*-hexane, 1 ml/min	Loop-type with FCSE	165 °C, He at 300 kPa	[63]
Acylglycerols in vegetable oil methyl esters	NPLC–GC–FID	100×2 mm Spherisorb S5W, *n*-hexane: CH_2Cl_2: ACN (79.95:20:0.05), 0.2 ml/min	Loop-type with FCSE	130 °C at 80 kPa	[58]
Stilbene hormones in beef	NPLC–GC–MS	25×2.1 mm Lichrospher Diol, MeOH:pentane (15:85), (90:10), 0.8 ml/min	On-column with FCSE	100 °C, He	[194]
Mineral oil PAHs in foods	LC–SE-LC–GC–FID	LC1: 25×4.6 mm Spherisorb Si5, pentane:CH_2Cl_2 LC2: Spherisorb NH5, pentane, 0.6 ml/min	In-line vaporiser	350 °C, H_2 at 70 kPa	[178,186]
Triglycerides in food	NPLCxGC–FID	25×2 mm AgSi, CH_2Cl_2:acetone gradient, 0.2 ml/min	PTV split	425 °C, He at 100 ml/min	[199]

Table 8
Biological applications

Sample	Method		Interface	Transfer conditions (temperature, carrier gas)	Refs.
DDE and PCBs in adipose tissue	NPLC–GC–ECD	50×1 mm Hypersil, *n*-hexane, 0.15 ml/min	On-column with PCSE	77 °C	[196]
β-Blockers in serum and urine	RPLC–LLE-GC–FID	20×2.1 CapcellPak C_{18} SG-120 (5 μm), 0.05 *M* boric acid: ACN (88:22) at pH 10.2, 0.8 ml/min	Loop-type interface with FCSE	92 °C, He 15.5 ml/min	[146]
Opiates in serum and urine	RPLC–LLE-GC–FID	20×2.1 CapcellPak C_{18} SG-120 (5 μm), 0.05 *M* boric acid:ACN (76:24) at pH 10.0, 0.8 ml/min	Loop-type interface with FCSE	90 °C, He at 1.5 bar	[147]

power of the two-dimensional system [47,50,51,182]. The number of samples to be analysed was high, and the traditional method was too laborious. For the non-polar fatty matrix, an NPLC method with silica column and hexane–MTBE as eluent was chosen to separate triglycerides from the analytes of interest. The loop-type interface with fully concurrent eluent evaporation could be applied because the analytes were not very volatile. The method that was developed allows the analysis of sterols, triterpene alcohols and wax esters in a single run (Fig. 6), something not possible by traditional methods. The ratio of the compounds acts as indicator of the treatment of the oil (cold-pressed or extracted). The traditional methods involve lengthy saphonication and clean up, which decrease the sample throughput severely. For its part, the LC–GC method eliminates most of the manual preparation work and provides more information on the sample and excellent accuracy with substantially reduced analysis time (15-fold). The investigation of minor lipid constituents also plays an important role in the assessment of authenticity of fats and oils. Sterols have proven to be a suitable class of compounds for deciding upon the genuineness of vegetable oils. Sterols are present as free alcohols as well as in esterified form, and analysis of the steryl esters allows characterisation of fats and oils. Adulteration of olive oil with hazelnut, almond or peanut oil, for example, can be detected by analysing the enantiomeric composition of γ-lactones in the oil by RPLC–GC using a chiral stationary phase in the GC step [125].

Radiation of food can be identified by measuring the radiation-induced hydrocarbons in the food. NPLC–GC has been employed to determine the irradiation products of fish, spices, chicken and eggs [8,22,23,29,127,179,180]. It has been shown that irradiation produces a number of characteristic radiolysis products of triglycerides, such as acids, propanediol esters, alkanes/alkanes, aldehydes and methyl esters [1,179]. In NPLC–GC, the triglycerides are removed by LC, and at the same time the components of interest are separated into their own fractions, which are then analysed by GC as shown in Fig. 7 [1,179]. NPLC separation using a silica column and a volatile eluent (pentane–MTBE) was chosen. Volatile eluent and in the transfer, an on-column interface with conventional retention gap technique was applied because the elution temperature of the most volatile analytes was low (120 °C).

As food matrices are very complex, a single LC step is not always sufficient for clean up of the sample before GC separation. Various food samples (rice, chocolate, edible oils, fish, canned food) have been analysed for mineral oil contaminants by an on-line NPLC-solvent evaporation (SE)-NPLC–GC system [177,178,186,197]. In the first LC step, a silica column and pentane–CH_2Cl_2 were used for separation, and in the second LC step an amino column with pentane was applied. In between the two LC steps, the volatile eluent from the first LC column was evaporated since the second amino bonded column did not tolerate it. The idea was to use the silica column for removal of fat and the

Table 9
Environmental applications

Sample	Method		Interface	Transfer conditions (temperature, carrier gas)	Refs.
Carbazole type PAHNs in air	NPLC–GC–NPD	50×4 mm Nucleosil $N(CH_3)_2$, MTBE at 1 ml/min	Loop-type with FCSE	73 °C, H_2 at 2 ml/min	[60]
Phthalates in water	RPLC–GC–FID	10×1 mm LiChrospher (5 μm), H_2O:MeOH (15:85), 0.7 and 0.1 ml/min	Vaporiser interface	PTV at 300 °C, He at 120 kPa, GC oven at 60 °C, He flow through SVE 600 ml/min	[115]
PAHs in fuels, combustion emissions and atmospheric samples	NPLC–GC–AED	10×2 mm Spherisorb, pentane: CH_2Cl_2 gradient, 0.1 ml/min	On-column with PCSE	65 °C, He at 120 Kpa	[27]
PAHs in soil	NPLC–GC–AED	150×2 mm silica (5 μm), *n*-hexane	Loop-type with FCSE	100 °C, He	[57]
PAHs in sediment	PHWE–NPLC–GC–FID	7×2.1. mm, Tenax TA, pentane:EtAC (9:1), 0.17 ml/min	On-column with PCSE	32 °C, He at 150 kPa	[33]
Brominated flame retardants in sediment	PHWE–NPLC–GC–FID	15×3 mm Luna cyano, pentane:EtAC (85:15) at 0.25 ml/min	On-column with FCSE	80 °C, He at 120 kPa	[35]
Organic acids in aerosol particles	SFE–NPLC–GC–MS	Pentane:EtAC (85:15) at 0.25 ml/min	On-column with PCSE	43 °C, He at 1.3 bar	[34]
PAHs in aerosol particles	SFE–NPLC–GC–MS	Pentane:EtAC (85:15) at 0.25 ml/min	On-column with PCSE	43 °C, He at 1.5 bar	[36]
BTEX in water	RPLCxGC–FID	30×2.1 mm ZirChrom PBDl, water, 0.5 ml/min	Drop interface	GC oven at 35 °C	[198]
Pesticide residues in water	RPLC–GC–NPD	25×4.6 mm Spherisorb ODS2, methanol, 1 ml/min	Through oven transfer adsorption desorption	Interface at 80 °C, He at 1800+900 ml/min	[121,123]
PAHs in chainsaw exhaust	NPLC–GC–FID	75×4 mm Nucleosil NO_2, pentane, 1 ml/min	Loop-type with FCSE	54 °C, He at 0.85 kPa	[192]

Table 10
Petrochemical applications

Sample	Method	LC conditions (column, eluent, flow-rate)	Interface	Transfer conditions (temperature, carrier gas)	Refs.
Sulfur compounds in middle distillates	NPLC–GC–SCD	250×2 mm aminosilica, *n*-heptane, 0.2 ml/min	On-column with PCSE	104 °C, N_2 at 1.2 ml/min	[190]
Carbazole and acridine type PANHs in coal	NPLC–GC–	75×4 mm Nucleosil 5NO_2, pentane, 1 ml/min	Loop-type with FCSE	55 °C, H_2 at 0.9 bar	[61]
PAHs in bitumen and bitumen fumes	FIA–NPLC–GC–FID	25×2 mm Chromegabond, THF:*n*-octane, 0.25 ml/min	Loop-type with FCSE	120 °C, He at 64 kPa	[59]
Sulfur containing compounds in diesel fuel	NPLC–GC–AED	150×2 mm Spherisorb Si5, *n*-pentane: CH_2Cl_2 gradient, 0.15 ml/min	Loop-type with FCSE	76 °C, He, 1.5 kPa	[62]
PAHs and aliphatics in crude oil distillation	SEC–NPLC–GC–FID	SEC: 25×4.6 mm PL-gel Minimix E, THF:*n*-decane (96:4), 0.5 ml/min and NPLC: 25×2 mm amino bonded silica, and *n*-heptane:ACN (99:1), 0.175 ml/min	On-column with PCSE	101 °C, He at 100 kPa	[26]
PAHs and aliphatics in oil residues	μ-NPLC–GC–FID	30×0.32 mm Spherisorb NH_2, *n*-hexane, 3.5 μl/min	In-column	50 °C, H_2 8 ml/min	[99]

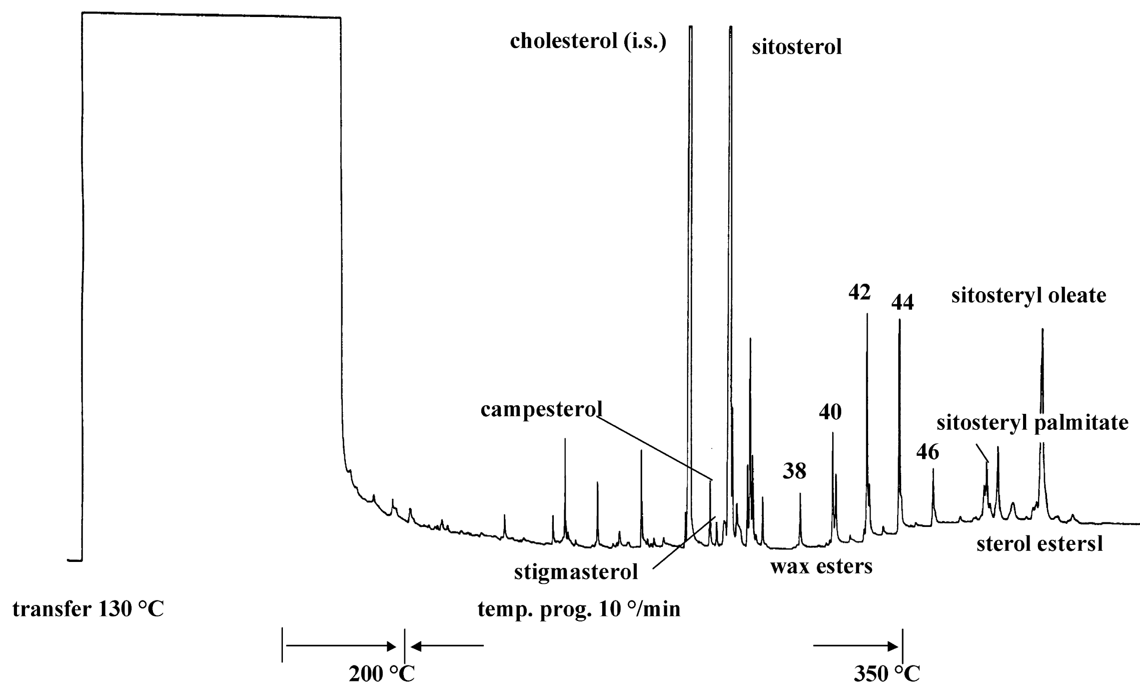

Fig. 6. NPLC–GC analysis of olive oil. Wax esters are indicated by the total number of carbon atoms. The moderately high concentration of the wax esters indicates that a small proportion of extraction oil has been added to the olive oil. Low concentration of free sitosterol (740 ppm) points towards pretreatment with an adsorbent (from Ref. [184]).

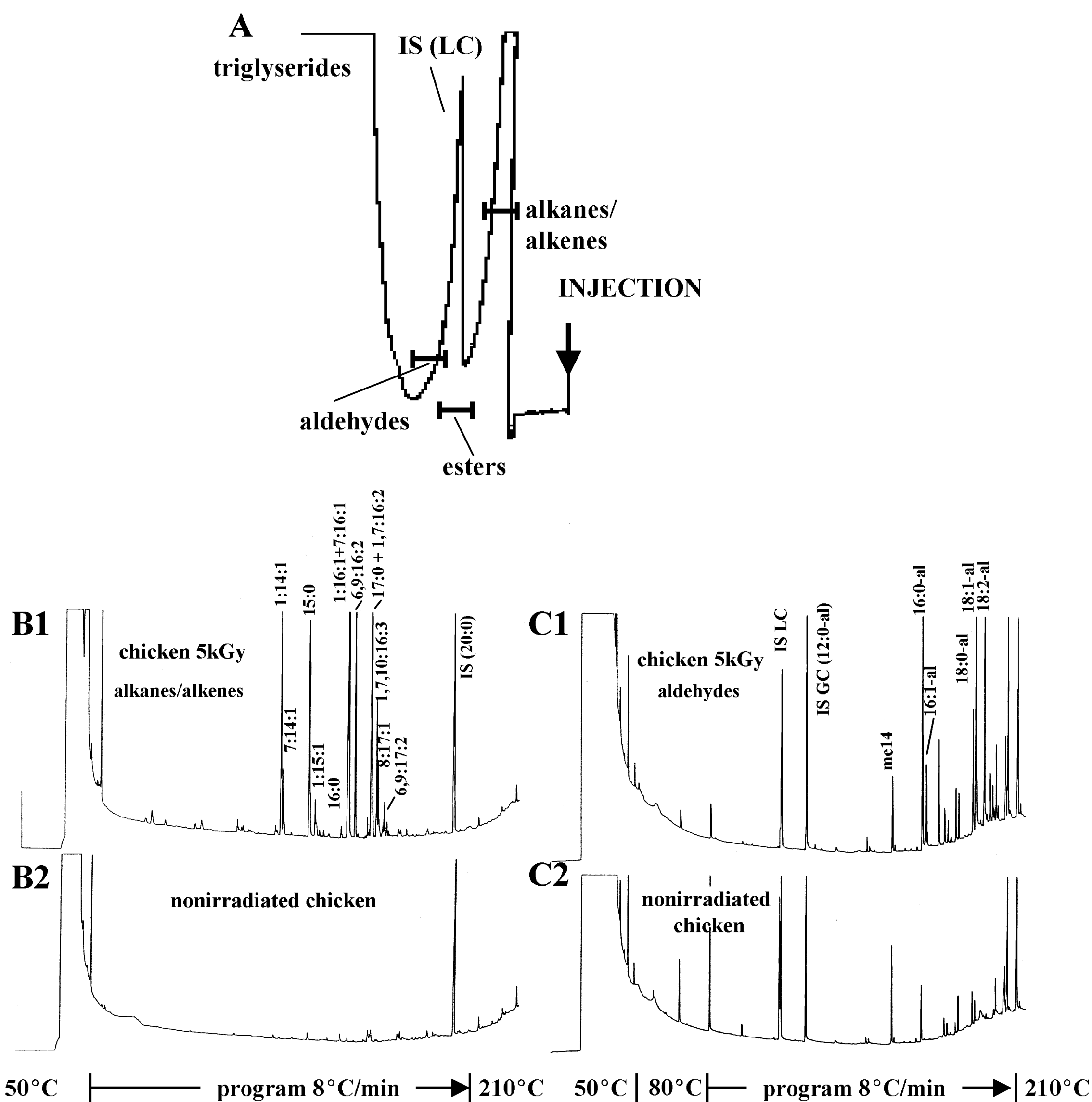

Fig. 7. Determination of irradiation of chicken by the radiolysis products of fat using NPLC–GC with on-column interface and partially concurrent solvent evaporation. Alkane/alkane and aldehyde fractions of the fat extracted from non-irradiated chicken and from chicken irradiated with 5 kGy (from Ref. [9]).

second amino column for fractioning the polyaromatic hydrocarbons according to ring size. An in-line vaporiser interface was used in the LC–GC coupling and it allowed the analysis of analytes ranging from C_{13} to C_{30}.

6.2. Petrochemical and industrial samples

LC–GC is particularly well suited for the analysis of petrochemical samples, which contain such a large number of analytes that it is difficult or even

impossible to separate them in a simple analytical system. NPLC–GC methods have been applied to group-type analyses of aliphatics and aromatics [6,14,19,26,189], the characterisation of coal liquids and fuels [59] and the determination of PAHs in solvent-refined coal, petroleum fractions and fuel and diesel particulate matter [99]. Other industrial samples have been investigated for polymers and their additives [52,98].

LC–GC is ideally suited for the analysis of petroleum and oil samples, since the sample can be fractionated in the LC part and the several fractions can then be transferred successively to GC. For example, oil is easily fractionated to aliphatic and aromatic groups, and the aromatic group can be fractionated further by ring size. An example of group-type analysis is the determination of sulphur-containing compounds in middle distillation fractions of oils by NPLC–GC [190]. The LC column (amino) was used to separate the analytes to fractions, the first one containing thiols, sulphides and thiophenes, the second benzothiophenes, the third dibenzothiophenes and the fourth benzonaphthothiophenes. Because the analytes were not very volatile, heptane could be used as the eluent in LC and rather high temperature could be used in the transfer (on-column with PCSE) (104 °C). The four fractions were then separated successively by GC. With GC alone the separation would be impossible.

6.3. Environmental applications

The environmental applications of LC–GC and SPE–GC include the determination of pesticides and various organic pollutants in water [10,44,68,121,124,191]; alkylated, oxygenated, and nitrated polycyclic aromatic compounds in air [60,61]; alkylated, oxygenated and nitrated PAHs in air particulates [21]; PAHs in sediment [57], air [27,48,49] and chainsaw exhaust [192]; and PCBs in sediment [97]. Both NPLC–GC and RPLC–GC methods have been developed. RPLC–GC has been utilised in particular for the analysis of aqueous samples. A typical environmental approach is SPE–GC, which is actually a simplified version of RPLC–GC, and it has been used to analyse water samples for herbicides, pesticides and PCBs [128–142]. A large volume of water sample is loaded to the SPE

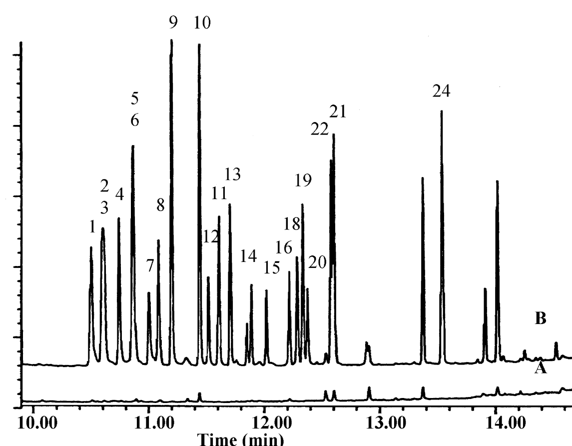

Fig. 8. Analysis of microcontaminants in river water with SPE-GC–MS using drying cartridge. Peaks: (1) 3,4-dichlorobenzeneamine, (2) dimethyl-phthalate, (3) 1,3-dinitrobenzene, (4) 4-butoxyphenol, (5) acenaphthene, (6) 3-nitroaniline, (7) 1-naphthenolol, (8) pentachlorobenzene, (9) 2,5-diethoxyaniline, (10) diethylphthalate, (11) 1-nitro-naphthalene, (12) 2,3,4,5-di-*O*-isopropyldiene, (13) tri-butylphosphate, (14) trifluoralin, (15) 1,4-dibutoxybenzene, (16) hexa-chlorobenzene, (18) simazine, (19) atrazine, (20) trichloroethylphosphate, (21) phenanthrene, (22) diazinon (I.S.), (23) caffeine and (24) metochlor (from Ref. [136]).

cartridge, the cartridge is dried and the analytes are desorbed with a small volume of organic solvent (usually methyl or ethyl acetate) into the GC. The determination of microcontaminants in river water by a SPE-GC–MS method is shown in Fig. 8 [136].

RPLC–GC with direct transfer using a vaporiser-gas discharge-precolumn solvent split interface has been employed for the determination of phthalates in water samples [115]. RPLC, with a short C_{18} column and H_2O–MeOH mixture as eluent, was used for concentration and clean-up of the sample. The sensitivity of the analysis was excellent due to the large sample volume (10 ml) and selective detection with MS. With a careful optimisation of transfer conditions, the aqueous LC eluent could be directly transferred to GC (Fig. 9). As a consequence of the transfer technique, the method was restricted to relatively non-volatile analytes, dibutylphthalate being the most volatile phthalate that could be quantitatively analysed.

Another example of environmental analysis is the determination of PAHs in urban air particulate extract by NPLC–GC [21]. Particles in urban air

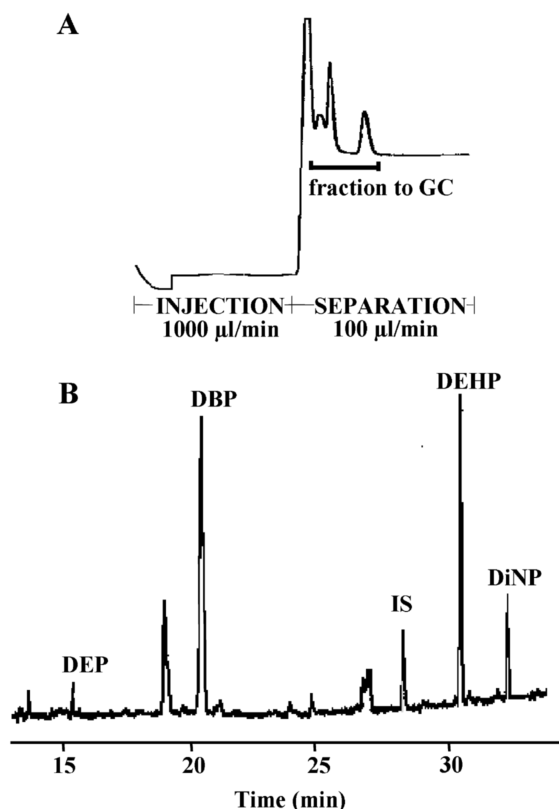

Fig. 9. RPLC–GC–MS analysis of 10 ml of water containing 55 ng/l of dibutylphthalate (DBP) and 44 ng/l of diethylhexylphthalate (DEHP). Diethylphthalate (DEP) and diisononylphthalate (DiNP) were not quantified (from Ref. [115]).

were collected with a high-volume sampler equipped with quartz filters. The particulates were extracted with SFE, collected to an organic solvent (dichloromethane) and injected to the LC–GC. Because part of the analytes were volatile, a volatile eluent (pentane–CH_2Cl_2) was used in the LC separation (silica column) and the transfer was performed via on-column interface applying partially concurrent eluent evaporation. The removal of interfering material by LC allowed improved detection of trace species.

6.4. Pharmaceutical and biological samples

LC–GC has been applied to the analysis of a number of pharmaceutical analytes, including heroin metabolites in urine [40], β-blockers and opiates in serum and urine [145–147,193], PCBs in plasma [54], stilbene hormones in beef [194] and DDEs and PCBs in adipose tissue [195]. Both NPLC–GC and RPLC–GC methods have been employed, and SPE–GC methods have been used in the analysis of biological fluids for drugs, such as benzodiazepenes in plasma [133] and steroid hormones in urine [142].

An RPLC–LLE–GC system has been developed for the analysis of β-blockers in human serum and urine [145,146]. The system enabled direct injection of the biological fluids to the RPLC–GC. In-direct technique (LLE) was used in the LC–GC coupling, because on-line derivatisation of the analytes was required under non-aqueous conditions. Derivatisation of the polar analytes was also accomplished on-line, by co-injection of silylation reagent during the transfer of the analyte fraction via a loop-type interface. Fully concurrent eluent evaporation was used during the transfer because the analytes were not particularly volatile. The total analysis time (less than 45 min) was considerably less than with traditional methods (2–3 h). A similar method has been utilised for the determination of opiates in urine samples [147].

NPLC–GC is the obvious choice for fat-containing biological samples such as tissue samples, since such samples usually require extraction with organic solvent. LC–GC is also well suited for analyses where only a limited amount of sample is available (e.g., patient tissue samples). An NPLC–GC method was developed for the analysis of tissue samples for DDE and PCBs in a multi-centre control study of breast cancer [196]. Clean-up of the organic extract was performed with NPLC using a silica column and hexane as the eluent. Hexane could be chosen as eluent because the analytes were not volatile. An on-column interface with PCSE was applied in the transfer. A chromatogram of tissue extract is shown in Fig. 10. Conventional methods for this kind of determination involve several steps, including collection and extraction of xenobials, removal of coextractives by appropriate clean-up methods using alumina, florisil, silica or GPC, and finally the analysis. For a large-scale study such as this, where the sample amount was limited to 200–800 µl, the conventional methods would have been too tedious and of too low sensitivity.

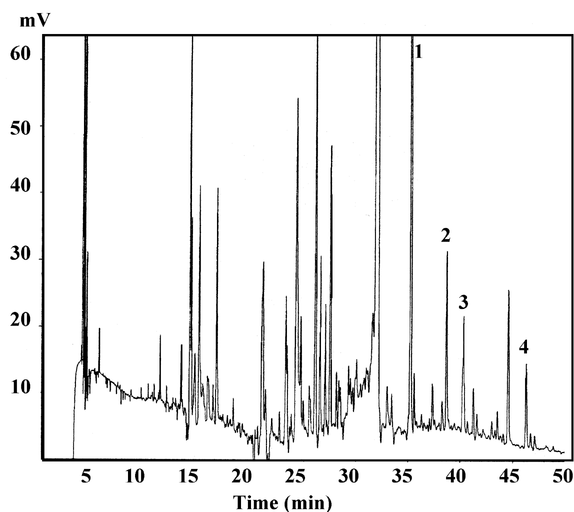

Fig. 10. Analysis of adipose tissue extract with NPLC–GC. Peaks: (1) *p*,*p*′-DDE, (2) PCB 153, (3) PCB 138 and (4) PCB 180 (from Ref. [196]).

6.5. Comprehensive LC×GC

In comprehensive LC×GC, all fractions eluting from the LC are analysed by GC. The comprehensive approach requires careful optimisation of the conditions. Especially the GC part has to be fast to keep the total analysis time reasonable. In addition, the solvent vapours should be evaporated and rapidly removed in the interface. As described below, two different approaches have been developed for comprehensive LC×GC [198,199].

A comprehensive LC×GC method has been developed for enhanced headspace analysis of water for volatile analytes [198]. The interface consisted of a chamber through which the aqueous LC effluent dropped. A carrier gas purged through the chamber carried volatile analytes from the droplets to GC. The LC analysis took only 5 min, and the GC analysis in a very short polar column took 2 s. Such fast analysis was possible because the sample was relatively clean and only five compounds of interest were separated. In total, 150 LC fractions could be analysed during one run [198].

An NPLC×GC method has been developed for the analysis of triglycerides in food samples, with PTV in split mode used as the interface [199]. A silver-loaded silica column with volatile eluent (methylene chloride with 0.5% ACN) was used in LC separation. The PTV was maintained at constant high temperature to allow fast vaporisation of the volatile LC fractions. A fast GC analysis method was developed to minimise the total analysis time. With a use of a short wide-bore GC column (5 m×0.53 mm I.D.), high gas flow (20 ml/min) and ballistic heating (1.2 °C/s), the total GC analysis time was less than 6 min. Altogether 125 fractions were collected from the LC, which was operated in stop-flow mode, and the total analysis time was 11 h. With traditional methods the analysis time would be substantially longer.

6.6. Coupling an extraction system on-line to LC–GC

Often extraction is required before the analysis. With solid samples, this is typically the first step of the analytical procedure. Where samples are complex, clean up of the extract is usually required, because the extract typically contains matrix compounds that may co-elute with the analytes of interest and obscure the quantitative analysis. It is relatively easy to couple an extraction system, such as on-line LLE, a membrane extraction technique, SFE or PHWE, on-line to LC–GC [59,34–36]. On-line coupling of an extraction method to LC–GC offers several advantages. The whole analysis, i.e., extraction, extract clean up and concentration and final separation, can be done in a closed on-line system. Since the whole extract can be utilised in the analysis, the sensitivity of the analysis is significantly increased. Furthermore, risks of sample loss and contamination are eliminated and consumption of organic solvents is minimised.

A recent study on the determination of mutagenicity and carcinogenicity markers in bitumen and bitumen fumes applied on-line liquid–liquid extraction–NPLC–GC [59]. The samples were injected to a stream of cyclohexane, which was mixed with a dimethyl sulphoxide (DMSO) stream in a segmentor. After extraction, the two phases were separated in a sandwich-type separator, and the DMSO extract was transferred to NPLC for separation of the three- to six-ring aromatics from higher aromatics, which were not of interest and which would have caused severe fouling of the GC system. Nitroaromatic-

substituted silica phase was used in LC separation because it enabled class-separation of the aromatics according to their ring size. Tetrahydrofuran–octane eluent was used in LC, and the transfer was performed by loop-type interface in which the THF evaporated fully concurrently while the octane acted as co-solvent and evaporated only partially during the transfer. In this way volatile analytes could be analysed too. The new method required only a few milligrams of sample and also enabled the analysis of bitumen fumes. This was not possible with the conventional method. The results were more accurate as well, since the heavier aromatics, which show very little or no mutagenicity or carcinogenicity, disturbed the analysis by conventional method.

An example of on-line extraction and analysis of solid samples is a PHWE–LC–GC system, applied to the analysis of PAHs and brominated flame-retardants in sediment [33,35]. The analytes were extracted with hot pressurised water (300 °C, 120 bar) and trapped in a solid-phase trap. After drying of the trap the analytes were eluted to LC for clean-up and the final analysis was done with GC [35]. The method was quantitative and sensitive, and a considerably smaller amount of sample was required than in traditional methods. Also, the total analysis time was considerable shorter, being only 120 min. A similar approach in which SFE was coupled on-line with LC–GC has been applied to the analysis of organic acids and PAHs in aerosol particles [34,36,200].

7. Concluding remarks

On-line LC–GC has proven to be a very powerful technique, which during recent years has been successfully applied to many complex sample matrices. The NPLC–GC methods already available are fairly simple to use, and their performance is usually superior to traditional off-line methods. The sensitivity is typically many times better than in traditional methods, and LC–GC methods are thus well suited for applications where the amount of sample is small. Furthermore, far less sample pretreatment is required before the analysis. The on-column interface has been widely used, as it also allows the analysis of relatively volatile analytes. Because LC enables very efficient clean up of the sample, the transferred fraction is generally clean and contamination of the column inlet is not a problem. The on-column interface can also be used with concurrent solvent evaporation. Since the solvent vapours are diluted with carrier gas, the oven temperature can be below the pressure-corrected boiling point of the solvent so that the retention of volatile analytes is improved. If a loop-type interface is used, the oven temperature must be higher, restricting analysis of the volatiles.

On-line RPLC–GC has not yet become a routine technique despite some encouraging developments. For them work will be required for a breakthrough in this area. Compared with the indirect systems for RPLC–GC coupling, the primary advantage of direct ones is their simplicity. No phase switching is needed, which simplifies the methods and facilitates automation, and makes the methods attractive for routine analysis. The on-column interface with special retention gaps and stationary phases is not yet of practical use for direct RPLC–GC coupling. Further studies are needed to produce columns with deactivation and stationary phases rugged enough for routine analysis. The main limitation of the loop-type interface with concurrent solvent evaporation is that it is restricted to high-boiling analytes. Even though the use of co-solvent allows the analysis of relatively volatile analytes, it makes the optimisation more difficult, in both the LC and the GC part. The use of a PTV interface with suitable adsorbent enables the analysis of relatively volatile analytes, but the analysis of high-boiling compounds is then restricted. The vaporiser-precolumn solvent split-gas discharge interface provides a simple interface for RPLC–GC coupling. The technique is limited to relatively nonvolatile analytes, however. Adding a second PTV system before the SVE makes it possible to analyse both relatively volatile and high boiling analytes. Among the indirect solutions, the phase switching with SPE technique would seem to be most promising. The main benefit compared with phase switching with on-line LLE is the simplicity. No phase separator is needed, and also the preconcentration with SPE is more efficient than that with LLE.

On-line coupling of an extraction system to LC–GC extends the applicability of the LC–GC technique further, allowing the analysis of solid samples

in a closed system. The main advantages are the improved sensitivity and the efficient cleaning of the extract, even enabling the use of total ion monitoring in MS detection.

In summary, on-line LC–GC, irrespective of the approach selected, is not simply a coupling of two well-established techniques; some adaptation and optimisation as well as knowledge of the underlying principles are generally required as well. However, the benefits of the on-line coupling technique are clear and the time invested in optimisation of the conditions is quickly repaid in shorter analysis time, better reproducibility and improved detection limits.

References

[1] K. Grob, in: On-Line Coupled LC–GC, Hüthig, Heidelberg, Germany, 1991.
[2] J.A. Apfel, H. McNair, J. Chromatogr. 279 (1983) 134.
[3] R.E. Majors, J. Chromatogr. Sci. 18 (1989) 571.
[4] I.A. Fowlis, J. High Resol. Chromatogr. 13 (1990) 213.
[5] T.V. Raglione, J.A. Troskosky, R.A. Hartwick, J. Chromatogr. 409 (1987) 205.
[6] K. Grob, D. Fröchlich, B. Schilling, H.P. Neukom, P. Nägeli, J. Chromatogr. 295 (1984) 55.
[7] F. Munari, A. Trisciani, G. Mapelli, S. Testianu, K. Grob, J.M. Colin, J. High Resolut. Chromatogr. 8 (1985) 601.
[8] H.G. Schmarr, A. Mosandl, K. Grob, J. High Resolut. Chromatogr. 12 (1989) 721.
[9] M. Biedermann, K. Grob, W. Meier, J. High Resolut. Chromatogr. 12 (1989) 591.
[10] J.J. Vreuls, V.P. Goudriaan, U.A.Th. Brinkman, G.J. deJong, J. High Resol. Chromatogr. 4 (1991) 475.
[11] H. Hyvönen, T. Auvinen, M.-L. Riekkola, K. Himberg, J. Microcol. Sep. 4 (1992) 123.
[12] H. Sirén, H. Hyvönen, M. Saarinen, S. Rovio, M.-L. Riekkola, Chromatographia 34 (1992) 421.
[13] A.-J. Bulterman, J.J. Vreuls, R.T. Ghijsen, U.A.Th. Brinkman, J. High Resolut. Chromatogr. 16 (1993) 397.
[14] A. Trisciani, F. Munari, J. High Resolut. Chromatogr. 17 (1994) 452.
[15] L. Mondello, P. Dugo, K.D. Bartle, B. Frere, G. Dugo, Chromatographia 39 (1994) 529.
[16] L. Mondello, K.D. Bartle, P. Dugo, P. Gans, G. Dugo, J. Microcol. Sep. 6 (1994) 237.
[17] E.C. Goosens, D. de Jong, G. de Jong, U.A.Th. Brinkman, J. Microcol. Sep. 6 (1994) 207.
[18] E.C. Goosens, I.M. Beerthuizen, D. deJong, G.J. deJong, U.A.Th. Brinkman, Chromatographia 40 (1995) 267.
[19] J. Beens, R. Tijssen, J. Microcol. Sep. 7 (1995) 345.
[20] F. Munari, P.A. Colombo, P. Magni, G. Zilioli, S. Trestianu, K. Grob, J. Microcol. Sep. 7 (1995) 403.
[21] A.C. Lewis, R.E. Robinson, K.D. Bartle, M.J. Pilling, Environ. Sci. Technol. 29 (1995) 1977.
[22] G. Schulzki, A. Spiegelberg, K.W. Bögl, G.A. Schreiber, J. Agric. Food Chem. 43 (1995) 372.
[23] G. Schulzki, A. Spiegelberg, K.W. Bögl, G.A. Schreiber, Radiat. Phys. Chem. 46 (1995) 765.
[24] L. Mondello, P. Dugo, G. Dugo, K.D. Bartle, J. Chromatog. Sci. 34 (1996) 174.
[25] G.R. van der Hoff, R.A. Baumann, P. van Zoonen, U.A.Th. Brinkman, J. High Res. Chromatogr. 20 (1997) 222.
[26] J. Blomberg, E.P.C. Mes, P.J. Schoenmakers, J.J.B. van der Does, J. High Res. Chromatogr. 20 (1997) 125.
[27] A.C. Lewis, S.A. Askey, K.M. Holden, K.D. Bartle, M.J. Pilling, J. High Resolut. Chromatogr. 20 (1997) 109.
[28] C. Droz, K. Grob, Z. Lebensm. Unters. Forsch. 205 (1997) 239.
[29] G. Schulzki, A. Spiegelberg, K.W. Bögl, G.A. Schreiber, J. Agric. Food Chem. 45 (1997) 3921.
[30] G. Jongenotter, M.A.T. Kerkhoff, H.C.M. van der Knaap, B.G.M. Vandeginste, J. High Resolut. Chromatogr. 22 (1999) 17.
[31] T. Andersson, T. Hyötyläinen, M.-L. Riekkola, J. High Resolut. Chromatogr. 22 (1999) 261–264.
[32] E. Boselli, K. Grob, G. Lercker, J. Agric. Food Chem. 48 (2000) 2868.
[33] T. Hyötyläinen, T. Andersson, K. Hartonen, K. Kuosmanen, M.-L. Riekkola, Anal. Chem. 72 (2000) 3070.
[34] M. Shimmo, T. Hyötyläinen, K. Hartonen, M.-L. Riekkola, J. Microcol. Sep. 13 (2001) 202.
[35] K. Kuosmanen, T. Hyötyläinen, K. Hartonen, M.-L. Riekkola, J. Chromatogr. A 943 (2002) 113.
[36] M. Shimmo, H. Adler, T. Hyötyläinen, K. Hartonen, M. Kulmala, M.- Riekkola L, Atmos. Environ. 36 (2002) 2985.
[37] K. Grob, J.-M. Stoll, J. High Resolut. Chromatogr. 9 (1986) 518.
[38] K. Grob, Th. Läubli, J. High Resolut. Chromatogr. 10 (1987) 435.
[39] K. Grob, J. High Resolut. Chromatogr. 10 (1987) 297.
[40] I.L. Davies, K.D. Bartle, P.T. Williams, G.E. Andrews, Anal. Chem. 60 (1988) 204.
[41] F. Munari, K. Grob, J. High Resolut. Chromatogr. 11 (1988) 172.
[42] V. Häkkinen, M.M. Virolainen, M.-L. Riekkola, J. High Resolut. Chromatogr. 11 (1988) 214.
[43] K. Grob, H.G. Schmarr, A. Mosandl, J. High Resolut. Chromator. 12 (1989) 375.
[44] P. van Zoonen, R. van der Hoff, E.A. Hogendoorn, J. High Resolut. Chromatogr. 13 (1990) 483.
[45] J.J. Vreuls, G.J. deJong, U.A.Th. Brinkman, Chromatographia 31 (1991) 113.
[46] K. Grob, A. Artho, P. Le Donne, J. High Resolut. Chromatogr. 15 (1992) 71.
[47] K. Grob, A. Artho, C. Mariani, Fat Sci. Techol. 10 (1992) 394.
[48] C. Östman, A. Bemgård, A. Colmsjö, J. High Resolut. Chromatogr. 15 (1992) 437.
[49] C. Östman, U. Nilsson, J. High Resolut. Chromatogr. 15 (1992) 745.

[50] M. Biederman, K. Grob, C. Mariani, Fat Sci. Techol. 4 (1993) 127.
[51] A. Artho, K. Grob, C. Mariani, Fat Sci. Techol. 5 (1993) 176.
[52] J. Blomberg, P.J. Schoenmakers, N.J. van der Hoed, J. High Resolut. Chromatogr. 17 (1994) 411.
[53] C. Östman, V. Nilssen, J. High Resolut. Chromatogr. 18 (1995) 685.
[54] E. Grimvall, C. Östman, U. Nilsson, J. High Resolut. Chromatogr. 18 (1995) 685.
[55] F. Modeste, M. Caude, P. Devaux, J. High Resolut. Chromatogr. 19 (1996) 535.
[56] F. Lanuzza, G. Micali, G. Calabro, J. High Resolut. Chromatogr. 19 (1996) 444.
[57] C. Jia, X. Wang, Q. Ou, Anal. Comm. 34 (1997) 53.
[58] M. Lechner, C. Bauer-Plank, E. Lorbeer, J. High Resolut. Chromatogr. 20 (1997) 581.
[59] J. Blomberg, P.C. de Groot, H.C.A. Brandt, J.J.B. van der Does, P.J.J. Schoenmakers, Chromatographia 849 (1999) 483.
[60] P. Tollbäck, H. Carlsson, C. Östman, J. High Resolut. Chromatogr. 23 (2000) 131.
[61] H. Carlsson, C. Östman, J. Chromatogr. 790 (1997) 73.
[62] K.M.L. Holden, K.D. Bartle, S.R. Hall, J. High Resolut. Chromatogr. 22 (1999) 159.
[63] J. Simas-Gandara, M. Sarria-Vidal, R. Rijk, J. AOAC Int. 83 (2000) 311.
[64] W. Kamm, F. Dionisi, L.-B. Fay, C. Hischenhuber, H.-G. Schmarr, J. Chromatogr. 918 (2001) 341.
[65] Th. Miller, H.-G. Frenzel, K.-H. Schmarr, in: P. Sandra (Ed.), Proc. 25th Int. Symp. on Capillary Chromatography (CD-ROM version), Naxos Software Solutions, Schriesheim, Germany, 2002, p. J15.
[66] K. Grob, J. High Resolut. Chromatogr. 13 (1990) 540.
[67] J. Staniewski, H.-G. Janssen, C.A. Cramers, J.A. Rijks, J. Microcol. Sep. 4 (1992) 331.
[68] H.G.J. Mol, H.-G.M. Janssen, C.A. Cramers, U.A.Th. Brinkman, J. High Resolut. Chromatogr. 16 (1993) 459.
[69] K. Grob, J. Chromatogr. A 703 (1995) 265.
[70] F.J. Senoráns, G. Reglero, M.J. Herraiz, Chromatogr. Sci. 33 (1995) 446.
[71] K. Grob, M. Bronz, J. Microcol. Sep. 7 (1995) 421.
[72] U. Boderius, K. Grob, M. Biedermann, J. High. Resolut. Chromatogr. 18 (1995) 573.
[73] K. Grob, M. Biedermann, J. Chromatogr. 750 (1996) 11.
[74] W. Engewald, J. Teske, J. Efer, J. Chromatogr. A 856 (1999) 259.
[75] M. Perez, J. Alario, A. Vazquez, J. Villén, J. Microcol. Sep. 11 (1999) 582.
[76] S. de Koning, M. van Lieshout, H.-G. Janssen, U.A.Th. Brinkman, J. Microcol. Sep. 12 (2000) 153.
[77] Th. Noy, E. Weiss, T. Herps, H. van Cruchen, J. Rijks, J. High Resolut. Hromatogr. 11 (1988) 181.
[78] G.S. Heo, J.K. Suh, J. High Resolut. Cromatogr. 13 (1990) 748.
[79] K. Grob, J. Chromatogr. 892 (2000) 407.
[80] E. Boselli, B. Grolimund, K. Grob, J. High Resolut. Chromatogr. 21 (1998) 355.
[81] B. Grolimund, E. Boselli, K. Grob, R. Amado, G. Lecker, J. High Resolut. Chromatogr. 21 (1998) 378.
[82] E. Boselli, K. Grob, G. Lecker, J. High Resolut. Chromatogr. 22 (1999) 327.
[83] T. Hyötyläinen, K. Grob, M.-L. Riekkola, J. High Resolut. Chromatogr. 20 (1997) 657–664.
[84] Th. Hankemeier, S.J. Kok, R.J.J. Vreuls, U.A.Th. Brinkman, J. Chromatogr. 811 (1998) 105.
[85] E. Boselli, K. Grob, G. Lecker, J. High Resolut. Chromatogr. 22 (1999) 149.
[86] M. Biedermann, K. Grob, M. Wiedmer, J. Chromatogr. A 764 (1997) 65.
[87] J. Staniewski, J.A. Rijks, J. Chromatogr. 623 (1992) 105.
[88] J. Staniewski, J.A. Rijks, J. High Resolut. Chromatogr. 16 (1993) 182.
[89] H.-G.J. Mol, P.J.M. Hendriks, H.-G. Janssen, C.A. Cramers, J. High Resolut. Chromatogr. 18 (1995) 124.
[90] H.-G.J. Mol, H.-G. Janssen, C.A. Cramers, U.A.Th. Brinkman, Trends Anal. Chem. 15 (1996) 206.
[91] J. Staniewski, H.-G. Janssen, C.A. Cramers, J.A. Rijks, J. Microcol. Sep. 4 (1992) 331.
[92] H.-G.J. Mol, M. Althuizen, H.-G. Janssen, C.A. Cramers, U.A.Th. Brinkman, J. High Resolut. Chromatogr. 19 (1996) 69.
[93] S. de Koning, M. van Lieshout, H.-G. Janssen, U.A.Th. Brinkman, J. Microcol. Sep. 12 (2000) 153.
[94] H.J. Cortes, E.L. Olberding, J.H. Wetters, Anal. Chim. Acta 236 (1990) 173.
[95] H. J Cortes, B.E. Richter, C.D. Pfeiffer, D. E Jensen, J. Chromatogr. 349 (1985) 55.
[96] D. Duquet, C. Dewaele, M. Verzele, M. McKinley, J. High Resolut. Chromatogr. 11 (1988) 824.
[97] F.A. Maris, E. Noroozian, R.R. Otten, R.C.J.M. van Dijk, G.J. de Jong, U.A.Th. Brinkman, J. High Resolut. Chromatogr. 11 (1988) 197.
[98] H. Cortes, G.E. Bormett, J.D. Graham, J. Microcol. Sep. 4 (1992) 51.
[99] T. Jiang, Y. Guan, J. Chromatogr. Sci. 37 (1999) 255.
[100] H.J. Cortes, C.D. Pfeiffer, G.L. Jewett, J. Microcol. Sep. 1 (1989) 28.
[101] E.C. Goosens, D. de Jong, J.H. M van der Berg, U.A.Th. Brinkman, J. Chromatogr. 552 (1991) 489.
[102] P. van der Hoff, K. van Zoonen, K. Grob, J. High Resolut. Chromatogr. 17 (1994) 37.
[103] A. Pouwelse, D. de Jong, J.H.M. van der Berg, J. High Resolut. Chromatogr. 11 (1988) 607.
[104] K. Grob, Z. Li, J. Chromatogr. 473 (1989) 381.
[105] K. Grob, Z. Li, J. Chromatogr. 473 (1989) 390.
[106] K. Grob, H.-P. Neukom, Z. Li, J. Chromatogr. 473 (1989) 401.
[107] K. Grob, A. Artho, J. High Resolut. Chromatogr. 14 (1991) 212.
[108] K. Grob, J. Chromatogr. 477 (1989) 73.
[109] K. Grob, E. Muller, J. Chromatogr. 473 (1989) 411.
[110] K. Grob, Z. Li, J. Chromatogr. 473 (1989) 4123.
[111] F.J. Senorans, M. Herraiz, J. Tabera, J. High Resolut. Chromatogr. 18 (1995) 433.

[112] F.J. Senorans, G. Reglero, M. Herraiz, Chromatogr. Sci. 33 (1995) 446.
[113] F.J. Senorans, J. Tabera, M. Herraiz, J. Agric. Food Chem. 44 (1996) 3189.
[114] K. Jauho, M.-L. Riekkola, J. Chromatogr. A 813 (1998) 113.
[115] T. Hyötyläinen, K. Grob, M. Biedermann, M.-L. Riekkola, J. High Resolut. Chromatogr. 20 (1997) 410–416.
[116] G.P. Blanch, M.L. Ruiz del Castillo, M. Herraiz, J. Chromatogr. Sci. 36 (1998) 589.
[117] F.J. Senorans, J. Villen, J. Tabera, M. Herraiz, J. Agric. Food Chem. 46 (1998) 1022.
[118] G.P. Blanch, J. Villen, M. Herraiz, J. Agric. Food Chem. 46 (1998) 1027.
[119] J. Villen, G.P. Blanch, M.L. Ruiz del Castillo, M. Herraiz, J. Agric. Food Chem. 46 (1998) 1419.
[120] M.L. Ruiz del Castillo, M. del Mar Caja, M. Herraiz, G.P. Blanch, J. Agric. Food Chem. 46 (1998) 5128.
[121] M. Perez, M.J. Alario, A. Vazquez, J. Villen, J. Microcol. Sep. 11 (1999) 582.
[122] G.P. Blanc, M. del Mar Caja, M.-L. Ruiz del Castillo, M. Herraiz, Eur. Food. Res. Technol. 210 (1999) 139.
[123] M. Perez, J. Alario, A. Vazquez, J. Villen, Anal. Chem. 72 (2000) 846.
[124] E. Pocurull, M. Biedermann, K. Grob, J. Chromatogr. A 876 (2000) 135.
[125] M.L. Ruiz del Castillo, M. Herraiz, G.P. Blanch, J. Agric. Food Chem. 48 (2000) 1186.
[126] M. del Mar Caja, M.-L. Ruiz del Castillo, R.M. Alvarez, M. Herraiz, G.P. Blanch, Eur. Food. Res. Technol. 211 (2000) 45.
[127] M. Perez, J. Alario, A. Vazquez, J. Villen, J. Microcol. Sep. 11 (1999) 582.
[128] J.J. Vreuls, V.P. Goudriaan, U.A.Th. Brinkman, G.J. de Jong, J. High Resolut. Chromatogr. 14 (1991) 475.
[129] J.J. Vreuls, R.T. Ghjisen, G.J. de Jong, U.A. Th Brinkman, J. Chromatogr. 625 (1992) 237.
[130] E. Noroozian, F.A. Maris, M.W.F. Nielen, R.W. Frei, G.J. de Jong, U.A.Th. Brinkman, J. High Resolut. Chromatogr. 10 (1987) 17.
[131] J.J. Vreuls, W.J.G.M. Cuppen, E. Dolecka, F.A. Maris, G.J. deJong, U.A.Th. Brinkman, J. High Resolut. Chromatogr. 12 (1989) 807.
[132] J.J. Vreuls, W.J.G.M. Cuppen, G.J. deJong, U.A.Th. Brinkman, J. High Resolut. Chromatogr. 13 (1990) 157.
[133] A. Farjam, J.J. Vreuls, W.J.G. M Cuppen, G.J. de Jong, U.A. Th. Brinkman, Anal. Chem. 63 (1991) 2481.
[134] P.J.M. Kwakman, J.J. Vreuls, U.A.Th. Brinkman, R.T. Ghijsen, Chromatographia 34 (1992) 41.
[135] Y. Pico, J.J. Vreuls, R.T. Ghijsen, U.A.Th. Brinkman, Chromatographia 38 (1994) 461.
[136] Th. Hankemeier, A.J.H. Louter, J. Dalluge, R.J.J. Vreuls, U.A.Th. Brinkman, J. High Resolut. Chromatogr. 21 (1998) 450.
[137] A.J.H. Louter, J. van Doornmalen, J. J Vreuls, U.A.Th. Brinkman, J. High Resolut. Chromatogr. 19 (1996) 679.
[138] J.J. Vreuls, U.A.Th. Brinkman, G.J. de Jong, K. Grob, A. Arto, J. High Resolut. Chromatogr. 14 (1991) 455.
[139] A.J.H. Louter, C. A van Beekvelt, P.C. Montanes, J. Slobodník, J.J. Vreuls, U.A.Th. Brinkman, J. Microcol. Sep. 5 (1993) 303.
[140] Th. Hankemeier, S. Ramalho, S.J. Kok, J.J. Vreuls, U.A. Th Brinkman, J. Chromatogr. A 811 (1998) 117.
[141] A.J.H. Louter, E. Bosma, J.C.A. Scipperen, J.J. Vreuls, U.A. Th Brinkman, J. Chromatogr. B 689 (1997) 35.
[142] K.K. Verma, A.J.H. Louter, A. Jain, E. Pocurull, J.J. Vreuls, U.A.Th. Brinkman, Chromatographia 44 (1997) 372.
[143] P. Wessel, J. Ogorka, G. Schwinger, M. Ulmer, J. High Resolut. Chromatogr. 16 (1993) 708.
[144] J. Ogorka, G. Schwinger, G. Bruat, J. Ogorka, G. Schwinger, V. Seidel, J. Chromatogr. 626 (1992) 87.
[145] T. Hyötyläinen, R. Pilviö, M.-L. Riekkola, J. High Resolut. Chromatogr. 19 (1996) 439.
[146] T. Hyötyläinen, T. Andersson, M.-L. Riekkola, J. Chromatogr. Sci 35 (1997) 280.
[147] T. Hyötyläinen, H. Keski-Hynnilä, M.-L. Riekkola, J. Chromatogr. A 771 (1997) 360.
[148] B. Karlberg, S. Thelander, Anal. Chim. Acta 98 (1978) 1.
[149] J. Roeraade, J. Chromatogr. 330 (1985) 263.
[150] L. Nord, B. Karlberg, Anal. Chim. Acta 118 (1980) 285.
[151] E.C. Goosens, I.M. Beerthuizen, D. de Jong, G.J. de Jong, U.A.Th. Brinkman, Chromatographia 40 (1995) 267.
[152] E. Fogelqvist, M. Krysell, L.-G. Danielsen, Anal. Chem. 5 (1986) 1516.
[153] E.C. Goosens, R.G. Bunschofen, V. Engelen, D. de Jong, J.A.M. van der Berg, J. High Resolut. Chromatogr. 13 (1990) 438.
[154] C. De Ruiter, J.H. Wolf, U.A.Th. Brinkman, R.W. Frei, Anal. Chim. Acta 192 (1987) 267.
[155] T.V. Raglione, R.A. Hartwick, J. Chromatogr. 295 (1984) 49.
[156] L. Nord, B. Karlberg, Anal. Chim. Acta 164 (1984) 233.
[157] H.G.J. Mol, J. Staniewski, H.-G. Janssen, C.A. Cramers, R.T. Ghijsen, U.A.Th. Brinkman, J. Chromatogr. 630 (1993) 201.
[158] S. Blomberg, J. Roeraade, J. High Resolut. Chromatogr. 13 (1990) 509.
[159] H.G.J. Mol, H.-G. Janssen, C.A. Cramers, U.A.Th. Brinkman, J. Microcol. Sep. 7 (1995) 247.
[160] G. Goretti, M.V. Russo, E. Veschetti, Chromatographia 35 (1993) 653.
[161] T. Hyötyläinen, M.-L. Riekkola, J. Chromatogr. A 819 (1998) 13.
[162] E.C. Goosens, D. de Jong, G.J. de Jong, U.A.Th. Brinkman, Chromatographia 47 (1998) 313.
[163] L. Mondello, G. Dugo, K.D. Bartle, J. Microcol. Sep. 8 (1996) 275.
[164] H.G.J. Mol, H.-G. Janssen, C.A. Cramers, J.J. Vreuls, U.A.Th. Brinkman, J. Chromatogr. A 703 (1995) 277.
[165] E.R. Brouwe, S. Kofman, U.A.Th. Brinkman, J. Chromatogr. A 703 (1995) 167.
[166] J.J. Vreuls, G.J. deJong, R.T. Ghjisen, U.A.Th. Brinkman, J. AOAC Int. 77 (1994) 306.
[167] U.A.Th. Brinkman, J. Chromatogr. A 665 (1994) 217.
[168] K. Grob, J. Chromatogr. 626 (1992) 25.

[169] P. Van Xenon, E.A. Hogendoorn, G.R. van der Hoff, R.A. Baumann, Trends Anal. Chem. 11 (1992) 11.
[170] I.L. Davies, M.W. Raynor, J.P. Kithinji, K.D. Bartle, P.T. Williams, G.E. Andrews, Anal. Chem. 60 (1988) 683.
[171] F. Munari, K. Grob, J. Chromatogr. Sci. 28 (1990) 61.
[172] M.-L. Riekkola, J. Chromatogr. 473 (1989) 315.
[173] K. Grob, B. Scilling, J. High Resolut. Chromatogr. 8 (1985) 726.
[174] J.J. Vreuls, A.J.H. Louter, U.A. Th. Brinkman, J. Chromatogr. A 865 (1999) 279.
[175] L. Mondello, P. Dugo, G. Dugo, A.C. Lewis, K.D. Bartle, J. Chromatogr. A 842 (1999) 373.
[176] K. Grob, M. Biedermann, J. Egli, Z. Lebensm. Unters. Forsch. 193 (1991) 213.
[177] S. Moret, K. Grob, L.S. Conte, J. High Resolut. Chromatogr. 19 (1996) 434.
[178] K. Grob, M. Vass, M. Biedermann, H.P. Neukom, Food Addit. Contam. 18 (2001) 1.
[179] M. Biedermann, K. Grob, W. Meier, J. High Resolut. Chromatogr. 12 (1989) 591.
[180] A. Bendini, T. Galliana Toschi, G. Lecker, Z. Lebensm. Unters. Forsch. A 207 (1998) 214.
[181] L. Mondello, P. Dugo, G. Dugo, K.D. Bartle, J. Chromatogr. Sci. 34 (1996) 174.
[182] M. Biedermann, K. Grob, C. Mariani, Fett Wiss. Technol. 95 (1993) 127.
[183] G. Full, G. Krammer, P. Schreier, J. High Resolut. Chromatogr. 14 (1991) 160.
[184] K. Grob, M. Lanfranchi, C. Mariani, J. Chromatogr. 471 (1989) 397.
[185] M.L. Ruiz del Castillo, M. Herraiz, G.P. Blanch, J. Agric. Food Chem. 4 (2000) 1186.
[186] S. Moret, K. Grob, L.S. Conte, Z. Lebensm. Unters. Forsch. A 204 (1997) 241.
[187] J.J. Vreuls, G.J. de Jong, U.A.Th. Brinkman, Chromatographia 31 (1991) 113.
[188] G.-P. Blanch, M. del Mar Caja, M.L. Ruiz del Castillo, M. Herraiz, J. Agric. Food. Chem. 46 (1998) 3153.
[189] A. Trisciani, F. Munari, J. High Resolut. Chromatogr. 17 (1994) 452.
[190] J. Beens, R. Tijseen, J. High Resolut. Chromatogr. 20 (1997) 131.
[191] P. van Zoonen, E.A. Hogendoorn, G.R. van der Hoff, R.A. Baumann, Trends Anal. Chem. 11 (1992) 11.
[192] R. Magnusson, C. Nilsson, K. Andersson, B. Andersson, R. Gieling, K. Wiberg, C. Ostman, U. Rannug, Environ. Technol. 21 (2000) 819.
[193] V. Giansello, E. Benn, G. Figini, A. Gazzaniga, J. Chromatogr. 473 (1989) 343.
[194] W.W.C. Quigley, C.G. Fraga, R.E. Synovec, J. Microcol. Sep. 12 (2000) 160.
[195] G.R. van der Hoff, R. Hoogerbrugge, R.A. Baumann, U.A. Th. Brinkman, P. van Zoonen, Chromatographia 52 (2000) 433.
[196] S.M. Gort, R. van der Hoff, R.A. Baumann, O. van Zoonen, J.M. Martin-Moreno, P. vant Veer, J. High Resolut. Chromatogr. 20 (1997) 138.
[197] K. Grob, M. Huber, U. Boderius, M. Bronz, Food Addit. contam. 14 (1997) 83.
[198] C.G. Chappell, C.S. Creaser, M.J. Shepard, Analyst 122 (1997) 955.
[199] M.M. van Deursen, doctoral thesis, Eindhoven Technical University, The Netherlands, 2002.
[200] M. Shimmo, K. Saarnio, P. Aalto, K. Hartonen, T. Hyötyläinen, M. Kulmala, M.L. Riekkola, J. Atmos. Chem. (submitted).