

On-line Coupled LC–GC: Theory and Applications

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Introduction

High-resolution gas chromatography (HRGC) is the most suitable technique for the analysis of volatile compounds. If the sample is a complex matrix, direct GC analysis cannot provide sufficient resolution and a pretreatment of some kind is necessary. The technique of multidimensional (MD) chromatography is an approach capable of providing greater resolution. The most common use of MD separation is the pretreatment of a complex matrix in an off-line mode.

Liquid chromatography (LC) is frequently used as a pre-separation technique for the ease of collecting and handling liquids. This type of sample treatment is widely used because of its simplicity; however, it is recognized that off-line techniques can be time consuming, operationally intensive, and difficult to automate and reproduce. Moreover, the probability of affecting the integrity of a sample increases with the number of manipulations the sample is subjected to. The coupling of two or more chromatographic systems in an on-line mode offers the advantages of ease of automation and greater reproducibility, usually in a shortened analysis time. Also, the loss of sample material during preparation is significantly reduced. The disadvantages of the on-line system are operational difficulty, expensive initial set-up and relatively complicated interfaces.

MDGC is perhaps the most widely used technique, because of the mobile-phase compatibility between the two separating techniques. The limitations of MDGC are mainly related to the necessity of having relatively volatile analytes and a relatively clean sample. This technique also lacks the selectivity variation dependence on the mobile phase and is limited by the selectivity differences obtained using common GC stationary phases.

MDLC offers other advantages, such as mobile phases with different polarities and a wide range of selectivity differences. Disadvantages mainly result from the lack of universal or very sensitive detectors and mobile-phase incompatibilities.

MD LC–GC combines the selectivity of LC with the efficiency and sensitivity of GC. Coupling the two technologies is not a trivial matter because the two separation techniques operate in phases that are in two different physical states.

The introduction of a large amount of solvent into a GC column requires the use of special techniques to separate the solvent from the sample. This technique must selectively remove the solvent, leaving the solute in a sharp band at the entrance of the separation column. Ways to make the transfer fraction from the LC system compatible with the gas chromatograph

have been explored since the early 1980s. The first on-line LC–capillary GC system was described by Majors in 1980.¹ He determined atrazine in sorghum using a conventional LC connected to a GC autosampler used as the interface.

Between 1981 and 1989, studies were performed — mainly by Grob — on the two most important overloading effects in capillary GC obtained when increasing the sample volume transferred to the column: band broadening in space and solvent effects (solvent trapping and phase soaking).^{2–4} The concept of retention gaps was developed and uncoated column inlets were used to increase sample volumes in on-column injection.^{5,6} LC columns with a reduced internal diameter were introduced by Cortes et al. to allow smaller volumes to be transferred into the GC.⁷ In 1985 an improvement in the retention gap technique was proposed, which used the partial evaporation of the LC eluent during introduction into the uncoated GC column,⁸ whilst still maintaining solvent necessary to reconcentrate volatile components. At the same time, a fully concurrent solvent evaporation technique was introduced.⁹ In this technique, no liquid spreads into the column and the solvent trapping effects are lost. Consequently, early eluted peaks are drastically broadened.

In 1989 the first fully automated LC–GC instrument was commercially introduced. Since then, many hundreds of papers have been published based on the use of LC–GC systems, many of them related to the analysis of food matrices, petrochemical and industrial samples, environmental samples, and pharmaceutical and biological samples.

Transfer Techniques for LC–GC Coupling

The introduction of large amounts of solvent into GC requires the use of a special technique to separate the solvent from the

Table 1: LC–GC eluent evaporation techniques.

Retention gap technique (on column interface)	- conventional - partially concurrent solvent evaporation
Concurrent eluent evaporation (loop-type interface)	- conventional - with co-solvent trapping
Vaporizers	- PTV solvent split - vapour overflow

sample. This technique must selectively remove the solvent, leaving the solute in a sharp band at the entrance of the separation column. Several interfaces have been developed for the LC–GC coupling, but most early versions have been abandoned and only on-column, loop-type and vaporizer interfaces are commonly employed today.

The nature of the LC eluent greatly influences the choice of the transferring technique, as will be explained within the description of each section. Table 1 summarizes the concepts of eluent evaporation allowing transfer of a large LC fraction into the GC.

Normal-phase chromatography (NPLC) and size exclusion chromatography (SEC) are more easily coupled with GC than reversed-phase liquid chromatography (RPLC). With NPLC–GC coupling, the eluent is typically a non-polar, volatile solvent such as pentane, hexane or a mixture of these solvents with a more polar modifier (e.g., diethyl ether, methyl tert-butyl ether (MTBE), isopropanol). RPLC methods can be chosen when the sample is aqueous. The coupling with GC of RPLC with water-containing eluents creates several specific problems and it is not widely applied. However, direct and indirect transfer techniques for RPLC–GC have been developed and will be discussed in a separate section.

Retention-Gap Techniques

The retention-gap method represents the best approach when samples to be analysed contain highly volatile components. The fraction must be transferred at a temperature below the boiling point of the LC eluent, corrected for the current inlet pressure.

Volatile components are reconcentrated by the solvent effects, primarily solvent trapping, while high-boiling compounds are spread by band broadening in space. Two retention gap effects can reconcentrate the bands: phase-ratio focusing and cold trapping, generally known as stationary phase focusing effects. Phase-ratio focusing is based on the differences in the retention powers of the uncoated retention gap and coated separation column. Cold trapping occurs when the analytes reach an

analytical column maintained at a temperature too low for them to migrate, so they accumulate and concentrate until the oven temperature is increased sufficiently to allow them to move through the column.¹⁰

These effects refer to the concentration obtained with an uncoated inlet. In fact, the term retention gap means a column inlet of a retention power lower than that of the analytical column. Figure 1 shows a schematic of an on-column interface.

Together with solvent effects, another effect called phase soaking occurs. When a large volume of solvent vapour has saturated the carrier gas, the properties of the stationary phase can be altered by swelling (thicker apparent film). This increased retention power improves the retention of analytes eluting near the solvent.

The retention gap technique, because of the solvent effects briefly explained above, allows the analysis of compounds eluting immediately after solvent peak. As a result of the limited capacity of uncoated precolumns to retain liquids, the technique is only suitable for the transfer of relatively small

Figure 2: Determination of furan fatty acids. (a) LC trace of 2 mg of extra virgin olive oil; (b) LC trace of sample A spiked with 650 ppm of diMeF(9,5) methyl ester; (c) LC–GC–PID trace of the LC fraction reported in (a); (d) LC trace of 200 mg of cold-pressed soybean oil. C18:1Me, C18:2Me and C18:3Me are the methyl esters of oleic, linoleic and linolenic acid, respectively. DiMeF(9,5)=methyl 10,13-epoxy-11,12-dimethyloctadeca-10,12-dienoate; diMeF(11,5)=methyl 12,15-epoxy-13,14-dimethyleicosa-12,14-dienoate; diMeF(11,5:1) and diMeF(11:1,5) are the olefinic derivatives of diMeF(11,5) with one unsaturation on the side chain conjugated with the furan ring. Reprinted by permission of American Chemical Society.¹⁵

Figure 1: Schematic representation of an on-column interface.

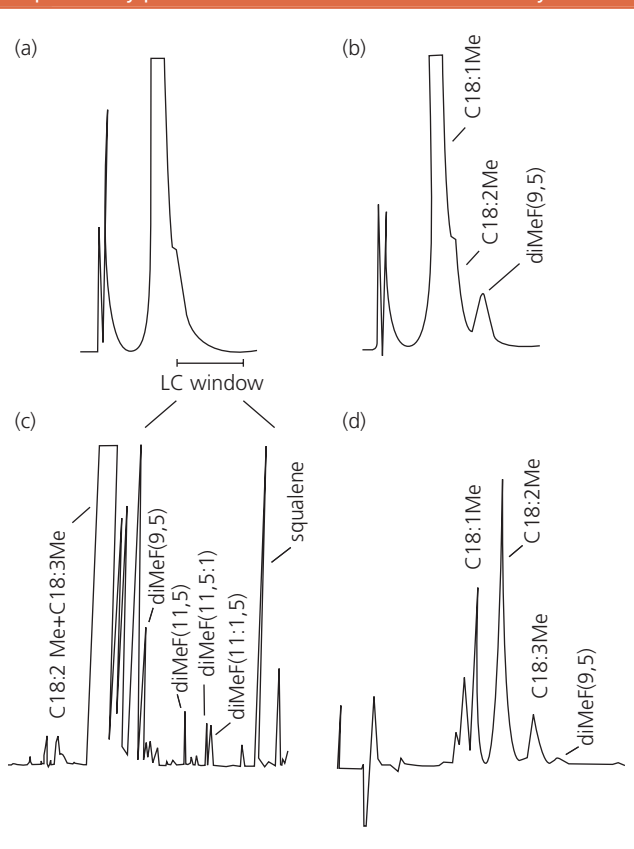
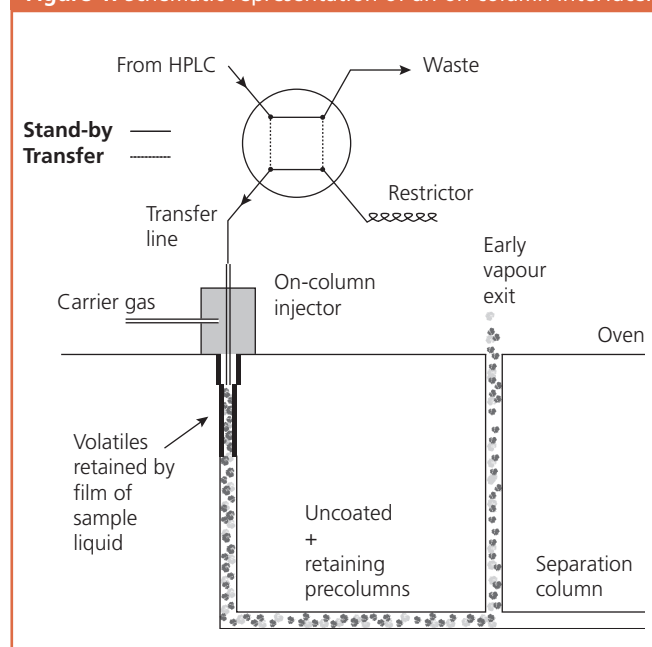


Table 2: LC–GC using on-column interface.

Sample	Method	LC conditions	Transfer conditions	Year (Ref.)
Brominated flame retardants	Pressurized hot water extraction (PHWE)–NPLC–GC–FID	Luna cyano 15 × 3 mm Pentane/Ethyl acetate (85/15) 0.25 mL/min	80 °C, He at 120 kPa	2002 (12)
PAHs in aerosol particles	Supercritical fluid extraction (SFE)–NPLC–GC–MS	Spherisorb cyano 100 × 2.1 mm Pentane/Ethyl acetate (85/15) 0.25 mL/min	43 °C, He at 1.5 bar	2002 (13)
Organic acids in aerosol	SFE–NPLC–GC–MS	Spherisorb cyano 100 × 2.1 mm Pentane/Ethyl acetate (85/15) 0.25 mL/min	43 °C, He at 1.3 bar	2001 (14)
Furan fatty acid in olive oil	NPLC–GC–PID	Lichrospher 250 × 2 mm, 5mm Hexane/MTBE (97.8/1.2) 0.5 mL/min	80 °C, H ₂	2000 (15)
PAHs in sediment	PHWE–NPLC–GC–FID	Tenax TA 7 × 2.1 mm Pentane/Ethyl acetate (90/10) 0.17 mL/min	32 °C, He at 150 kPa	2000 (16)

fractions; long retention gaps and long analysis times are needed, because the solvent must be completely evaporated prior to starting the elution of components of interest.

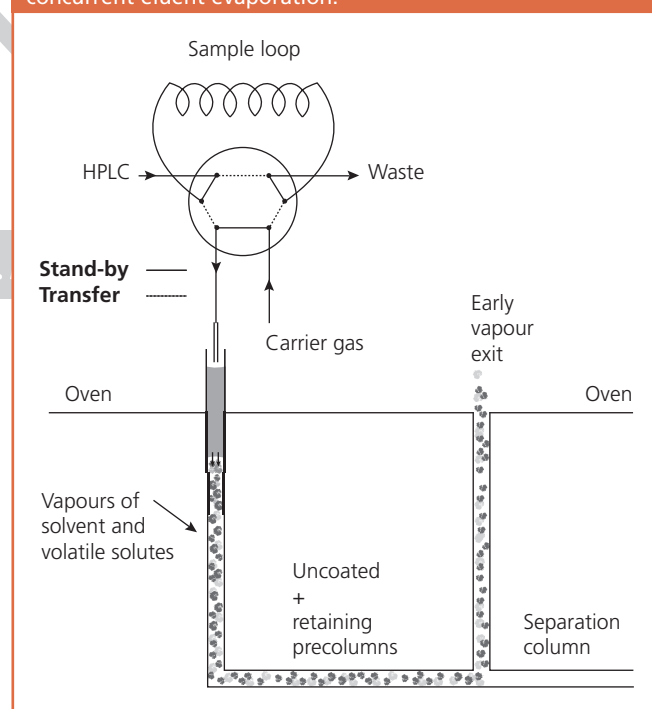
A technique called “partially concurrent eluent evaporation” has been introduced to overcome some drawbacks of the retention gap technique. Using this technique, part of the solvent is evaporated concurrently during its introduction into the GC. Applying this, the volume of the sample fraction can be greatly enlarged. Introducing an early solvent vapour exit (SVE) improves partially concurrent eluent evaporation. In theory, the SVE could be placed between the uncoated precolumn and the analytical column, but this makes the closure of the SVE critical for partial losses of early-eluting peaks. A section of the analytical column, a “retaining precolumn” was usually installed after the uncoated precolumn, but before the SVE.

The retention gap technique was introduced more than 15 years ago, but has recently received some substantial modifications, as reported by Grob in a review of 2000.¹¹ Some observations could not be explained by the classic theory postulating that solvent evaporation takes place exclusively at the rear of the flooded zone. The improvement of the retention gap technique was obtained through a higher capacity of the uncoated precolumn that enables the transfer of enlarged fractions (1000 µL). There is no need for a retaining precolumn, because it acted as a restriction. A restriction in the vapour outlet is equally effective.

Recent applications that use the classic retention gap for the transfer from HPLC to HRGC were not found, while several applications use retention gap with partially concurrent solvent evaporation, as summarized in Table 2.^{12–16}

As an example, Figure 2 shows the results of the determination of furan fatty acids in extra virgin olive oil.¹⁵ These components are present in the oil at very low levels (some ppm), so their analysis requires a very powerful method. In this instance, the extra virgin olive oil was transmethylated and then injected into the LC–GC system. The LC column was 250 × 2 mm i.d., packed with silica particles of 5 µm, and

operated at a flow-rate of 500 µL/min with MTBE (1.2%) in hexane. The on-column interface was a transfer line consisting of a 30 cm × 0.17 mm o.d. silica capillary tubing. The transfer volume was 1500 µL. The retention gap was a 10 m × 0.53 mm i.d. precolumn deactivated with an ultrathin layer of OV-1701 OH stationary phase. No retaining precolumn was used. The separation column was 30 m × 0.32 mm i.d., coated with an apolar stationary phase (0.2 µm film thickness). Hydrogen was used as carrier gas. The LC–GC transfer occurred at 80 °C. Usually, the separation methods used to

Figure 3: Schematic representation of a loop-type interface for concurrent eluent evaporation.

isolate furan fatty acids involve hydrogenation for a better separation between furan fatty acids and straight-chain fatty acids. These methods should be avoided because they cause the elimination of unsaturated furan fatty acids, which are natural components of some food lipids. The use of the photoionization detector (PID) improved qualitative and quantitative detection of furan fatty acids because saturated fatty acids were not ionized at the low ionization energy used, the response of unsaturated fatty acids was strongly attenuated in comparison with the FID trace, while response of furan fatty acids was amplified.

Loop-Type Interface with Concurrent Eluent Evaporation

Concurrent eluent evaporation is the most used technique because of its simplicity and the possibility of transferring large amounts of solvent. The fraction to be analysed is collected in a loop connected to a multiport valve. By switching the valve, the sample is driven by the carrier gas into the uncoated GC column, maintained at a temperature at or above the solvent boiling point. In this way, the liquid can be transferred at a speed corresponding to the evaporation speed. An early vapour exit is usually placed after a few metres of the deactivated precolumn. The SVE valve is opened during the transfer to reduce the amount of solvent reaching the detector and to increase the solvent evaporation rate. Figure 3 shows a loop-type interface scheme for fully concurrent solvent evaporation.

When the sample solvent evaporates at the front end of the liquid, volatile components co-evaporate with the solvent.

There is no solvent trapping, so volatile components can be lost through the SVE or coelute with the solvent. The first properly shaped peaks are eluted at temperatures only 40–120 °C above the transfer temperature, varying with the eluent and the sample volume.

Concurrent solvent evaporation using the loop-type interface is easy to handle, allows the transfer of very large volumes of HPLC eluent and renders solvent evaporation very efficient. Moreover, the requirement that the solvent wets the column is less important. There is no liquid flood in the GC column, and a virtually unlimited volume of liquid can be handled. Recent applications of the loop-type interface using fully concurrent eluent evaporation were developed for the study of both food and environmental samples, and are summarized in Table 3.^{17–23} As an example, Figure 4 shows the analysis of γ -oryzanol in rice lipids.¹⁷ γ -Oryzanol comprises a mixture of phytosteryl ferulates located in rice bran. Separation of individual γ -oryzanol components can be achieved by RPLC after a long purification step of the fraction. On-line LC–GC has been demonstrated to be an elegant and efficient approach for the analysis of minor constituents in complex matrices.

The main drawback of this technique is loss of volatile compounds. The partial solution to reduce the loss of volatile components during concurrent evaporation of the main eluent is to use the so-called “co-solvent trapping technique”. A small amount of higher boiling co-solvent is added to the main solvent to build up a layer of condensed liquid ahead of the main

Table 3: LC–GC using loop-type interface.

Sample	Method	LC conditions	Transfer conditions	Year (Ref.)
γ -Oryzanol in rice lipids	NPLC–GC–FID	Nucleosil 50-5 25 × 3 mm Hexane / isopropanol (98.5/1.5) 0.45 mL/min	60 °C, H ₂ at 75 kPa	2003 (17)
Stery esters in cocoa butter	NPLC–GC–FID	Zorbax-SB 150 × 2.1 mm, 5 μ m Dichloromethane/acetonitrile/ <i>n</i> -hexane (2/0.1/97.9) 0.2 mL/min	120 °C, He at 250 kPa	2002 (18)
Vegetable oils (β -sitosterol) in milk fat	NPLC–GC–FID	Hypersil 100 × 2.1 mm, 5 μ m <i>n</i> -hexane with 0.8% of 2-propanol 0.2 mL/min	120 °C, H ₂ at 200 kPa	2002 (19)
PCBs	NPLC–GC–FID	Hypercarb porous grafitic carbon 100 × 2.1 mm i.d., 5 μ m d_p Dichloromethane/benzene/ethylbenzene, 68 :28 :4 0.1 mL/min	85 °C, He at 2 bar	2002 (20)
Paraffins in food stimulants and packing materials	NPLC–GC–FID	Lichrospher SI60 25 × 4.6 mm, 5 μ m <i>n</i> -Hexane 1 mL/min	165 °C, He at 300 kPa	2000 (21)
Carbazole-type PAHs in air	NPLC–GC–NPD	Nucleosil N (CH ₃) ₂ MTBE 1 mL/min	73 °C, H ₂ at 2 mL/min	2000 (22)
PAHs in chainsaw exhaust	NPLC–GC–FID	Nucleosil NO ₂ Pentane 1 mL/min	54 °C, He at 0.85 kPa	2000 (23)

evaporation site, retaining volatile solutes by solvent trapping. The most frequently used co-solvents are alkanes (C7–C10) for organic solvents, and butoxy-ethanol for aqueous solvents.

Co-solvent trapping has never been used for routine analysis of normal-phase eluents because of the superiority of partially concurrent eluent evaporation with the on-column interface for such solvents. Co-solvent trapping was primarily investigated for transferring water-containing eluents. However, the big obstacle to developing this technique in reversed-phase LC–GC is that water renders the uncoated precolumn absorptive after a few transfers.

Vaporization with Hot Injector

Cold temperature programmed sample introduction is an attractive technique for introducing large volumes in capillary GC and for LC–GC interfacing. Many applications involving the vaporizer interface use a programmable temperature vaporization (PTV) or a hot vaporizing chamber packed with some inert material or an adsorbent.

There are several ways to perform the transfer. PTV with solvent trapping in packed beds can be used to transfer large

volumes into the GC as an alternative to on-column injection. This technique shows some advantages compared with capillary precolumns: packed beds retain more liquid per unit internal volume; wettability is not important for the retention of the liquid, and packing materials for the liners are more stable than deactivated silica tubing with water. Another advantage is that packed beds tolerate substantially more non-evaporating byproducts, but this is important for direct large-volume injection of extracts because LC pre-separates with an efficiency that usually delivers sufficiently clean fractions for on-column injection.

The disadvantages of this technique occur when thermally labile components must be analysed. In fact, because of the high retention power, the packed chambers must be heated above the column temperature to release the solutes.

In the PTV solvent split mode, the injection is made into a cool injector, and the solvent is removed via the split valve. After solvent evaporation the split valve is closed and the injection chamber is heated to transfer the solutes into the column. Liners are usually packed with Tenax TA and Thermo-trap TA. To increase trapping efficiency, liners with

Figure 4: Analysis of γ -oryzanol extracted from a rough rice sample using on-line LC–GC. (a) Separation of γ -oryzanol from other rice lipids by NPLC using UV detection at 200 and 290 nm, respectively. The transferred fraction is shown by the indicated time window. (b) GC chromatogram of the on-line transferred fraction containing: 1 = campesteryl ferulate; 2 = campestanil ferulate; 3 = β -sitosteril ferulate; 4 = cycloartenil ferulate; 5 = 24-methylenecycloartenil ferulate. For other conditions, see Table 3. Reprinted by permission of Elsevier.¹⁷

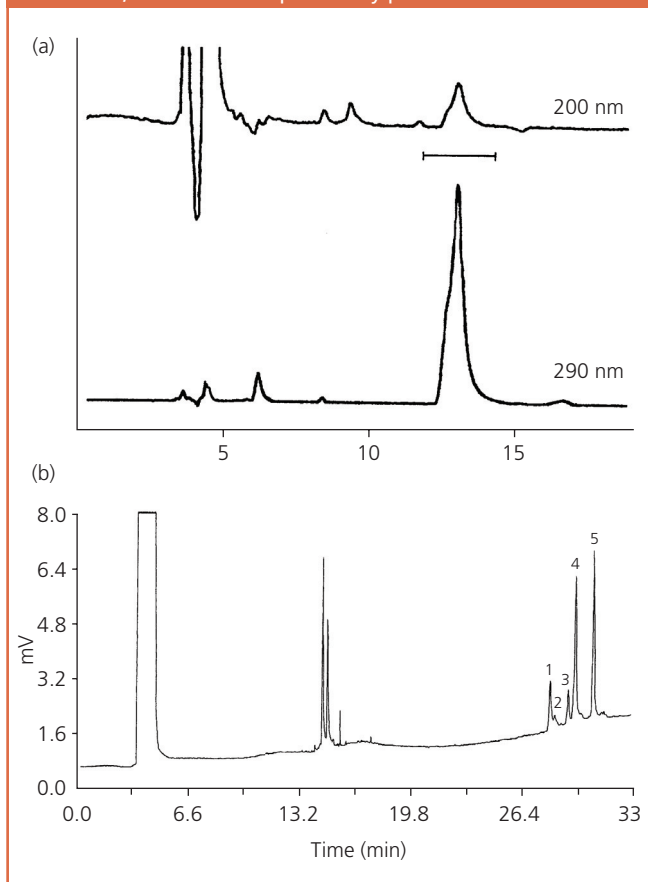


Figure 5: Coupling of LC to GC through PTV solvent splitting using an autosampler as interface.

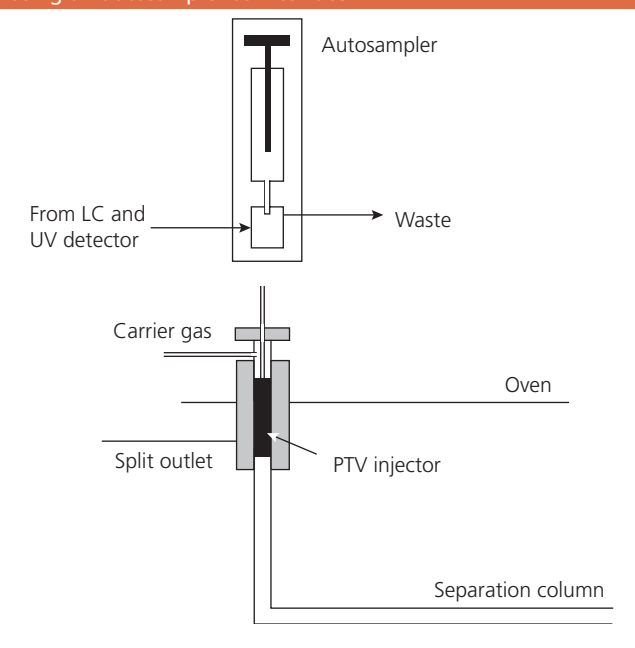
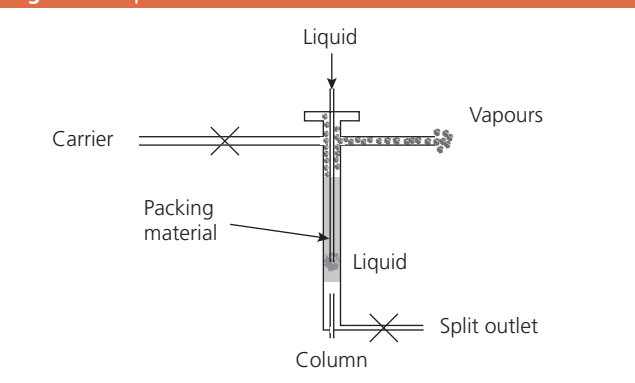


Figure 6: Vapour overflow interface.



sintered porous glass beads were introduced. Recently, a PTV-based LC–GC interface as a modular system has been introduced on the market. The interface shown in Figure 5, developed by Sandra and co-workers,²⁴ consists of a flowcell, in which the fraction is sampled by a large-volume autosampler and automatically injected into a PTV device using the solvent split mode.

Figure 6 shows a vapour overflow interface. In the PTV vapour overflow technique the liquid is introduced into a packed vaporizing chamber maintained above the solvent boiling temperature at a pressure that is near or below ambient. There is no carrier-gas flow during the transfer and vapours are discharged through the septum purge exit by expansion during evaporation (overflow). At the end of solvent evaporation, the septum purge is closed, the injector is warmed up and the trapped analytes are released into the column.

The PTV solvent split technique is suitable for medium and less volatile compounds, while the overflow technique is applicable only to less volatile compounds. An improvement in the overflow technique to the retention of more volatile components was presented by Grob (wire interface) in 1995²⁵ (see Figure 7). The vaporizer consisted of a transfer line of fused silica thermostatted at 250–350 °C. Inside the vaporizer a 5 cm length of steel wire or fused silica was inserted to prevent shooting of liquid from violent evaporation. The system is equipped with a retaining precolumn and an SVE. The carrier-gas flow is interrupted during transfer and vapours are discharged by overflow through the vapour exit. The oven is maintained at a low temperature (lower than with a loop-type interface) to improve retention of sample components by phase soaking. Table 4 summarizes the most recent applications developed using a vaporizer interface.^{26–29}

Transfer of Water-Containing Solvent Mixtures

RPLC–GC coupling is much more demanding than NPLC–GC, but is an attractive technique because a large number of samples to be analysed by GC have an aqueous matrix. The main drawbacks of direct injection of water-containing solvent mixtures in capillary GC are as follows:

condensed water destroys the deactivation of the precolumn because of the hydrolysis of siloxane bonds; the extremely high surface tension of water does not allow it to wet the deactivated capillary surface, essential for the formation of the solvent film that allows solute trapping; the evaporation rate is low; water has a high boiling point and molar vaporization enthalpy; and because of its low molecular weight water forms a very large volume of vapour per volume of liquid. Direct and indirect solutions have been proposed to couple RPLC to GC.

Direct transfer of RPLC eluents: The solutions developed for direct transfer of RPLC eluents are μ LC–GC coupling, retention-gap techniques using special deactivated retention

Figure 7: Wire interface for concurrent evaporation of organic solvent.

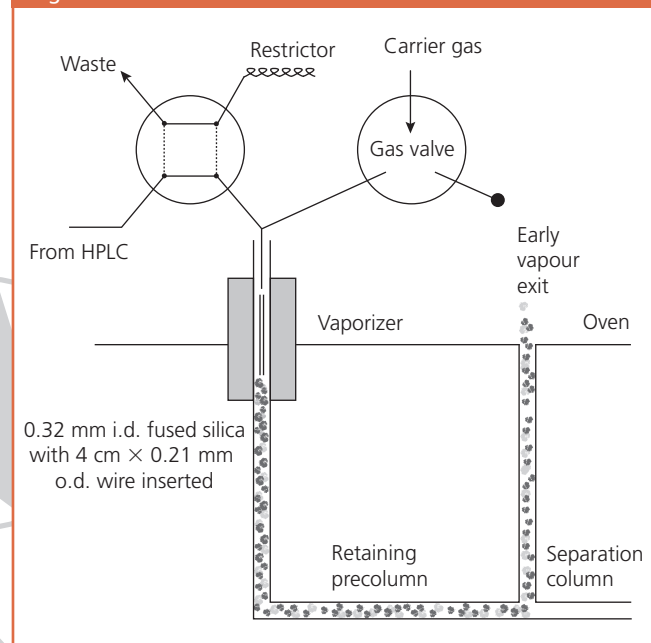


Table 4: LC–GC using vaporizer interface.

Sample	Method	LC-conditions	Transfer conditions	Year (Ref.)
Mineral oil PAHs in foods	LC–SE–LC–GC–FID	LC1: Spherisorb Si5 25 × 4.6 mm Pentane/dichloromethane	350 °C, H ₂ at 70 kPa	2001 (26)
		LC2: Spherisorb NH5 Pentane 0.6 mL/min		
Enantiomers of γ -lactones in edible oils	PLC–GC–FID	Kromasil-100-10C4 50 × 4.6 mm Water/methanol (10/90) 1.4 mL/min	21 °C, He at 1800 mL/min	2000 (27)
Pesticide residues in water	RPLC–GC–NPD	Spherisorb ODS2 25 × 4.6 mm Methanol 1 mL/min	Interface at 80 °C, He at 1800 + 900 mL/min	2000 (28)
Volatile compounds in edible oils	RPLC–GC–FID	Kromasil-100-10C4 50 × 4.6 mm i.d., T=45 °C Water/methanol 1.8 mL/min	21 °C, He at 1500 mL/min	2000 (29)

gaps, the loop-type interface and the vaporizer interface, and have been reviewed recently.³⁰

In μ LC the sample fractions are so small (10 μ L) that the low evaporation rate of water is not a problem. The disadvantage is the low sample capacity of the LC microcolumn.

Using a retention-gap technique, the main problem is poor wettability of the uncoated precolumn. Many studies have demonstrated that the transfer of aqueous solvents with the retention-gap technique was not achievable because it was not possible to find a precolumn that was at the same time both water-wettable and inert. A partial solution to this problem can be the use of an organic solvent with a higher boiling point than that of water, in a mixture with water, with phenyl- and cyanosilylated precolumns. Azeotropic mixtures of water and organic solvents, for example, water and *n*-propanol, 28:72, or water and acetonitrile, 16:84, can wet such precolumns, allowing the introduction of water by the retention-gap technique.

Concurrent solvent evaporation does not need good wettability, so the loop-type interface can be used for the transfer of aqueous solvents. The deteriorating effect of water vapour is far less pronounced than that of liquid water. However, because of the large amount of vapour released and the high temperature needed for concurrent solvent evaporation of water, and because of the absence of solvent effects, this technique is limited to high-boiling analytes. A partial solution to this problem is the use of the co-solvent trapping technique.

Several methods using a vaporizer interface for direct transfer of aqueous eluents have been proposed. A slightly modified PTV interface that allows the introduction of water-containing eluents is a "vaporizer chamber/precursor solvent split/gas discharge interface", shown in Figure 8. With this technique, the sample is driven into a vaporizer chamber maintained at a temperature suitable for solute evaporation, by the LC pump. The vapours are then removed through a retaining precolumn and an early vapour exit, driven by the flow of carrier gas (discharge). The drawback of this technique is that only high-boiling solute can be analysed, because the temperature of the precolumn must be sufficiently high to prevent recondensation of water. To overcome this problem, a modified system, called "swing", was developed³¹ (see Figure 9). In this system, a PTV injector was installed in the vapour outlet. For desorption, the gas flow was reversed, entering the packing with highest retention power and passing through the retaining precolumn into the separation column. In this way, the high-boiling

solutes are retained in a zone of rather low retention power (retaining precolumn), while the more volatile components are retained in the trapping material of the PTV with higher retention power. After the transfer and solvent evaporation, the direction of the carrier-gas flow (swing system) is modified, the PTV is used as inlet, as the PTV temperature rises above the oven temperature the trapped analytes are released and the analysis starts.

Indirect introduction of water: There are three main indirect methods used for introduction of aqueous solvents into a GC: solid phase extraction (SPE), on-line liquid-liquid extraction and open tubular trapping. A method for coupling RPLC to GC without introducing water into the GC uses precolumns or SPE cartridges. A large amount of water containing the analytes is passed through the precolumn for solute enrichment. Then the column is flushed with an inert gas to remove the residual water and the analytes eluted by an organic solvent. Drying, performed with purging of the cartridge with the inert gas, is time consuming. An interesting alternative is the use of a drying cartridge positioned between the SPE and GC units, and filled with a drying agent such as silica. This small cartridge can be reconditioned during the GC run by heating.

Sometimes, SPE with thermal desorption (SPETD) has been performed. In this instance, analytes are eluted from the stationary phase by heating the trap instead of using an organic eluent. The trap is often located directly at the GC injector.

The material used for packing the cartridge must withstand high temperatures, so different packing materials than those employed with SPE must be used. Tenax is a good material for SPETD because it presents good thermal stability, sufficient retention power for analytes and poor enough interaction with water to allow optimal drying.

Another method for phase switching is liquid-liquid extraction (LLE). In automated LLE the solvent (organic) is injected via a small segmentor into the water sample, which flows through a small-diameter fused-silica tube. After the extraction, phase separation is achieved by using a simple T-piece, a semipermeable PTFE membrane or a sandwich-type phase separator.

The analysis of organic compounds from aqueous samples is also possible by using open tubular trapping (OTT) columns. The extraction of aqueous samples is performed in open tubular traps (5 m \times 0.53 mm i.d.), coated with a thick film of silicone stationary phase. Removal of water is achieved by

Figure 8: Vaporizing chamber/precursor solvent-split/gas discharge interface.

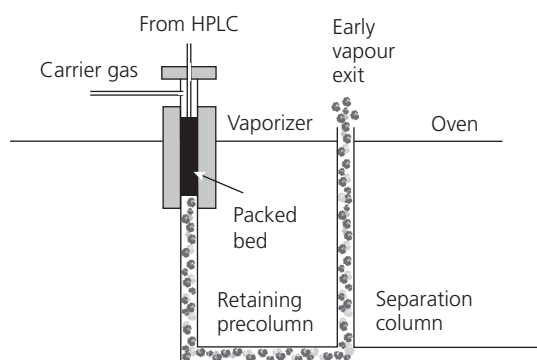
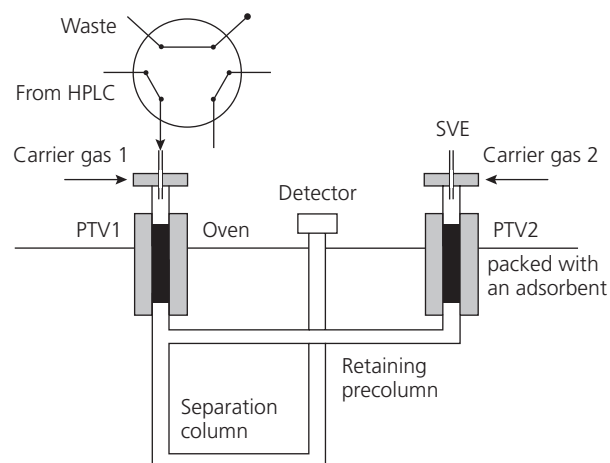


Figure 9: Swing interface.



purging the trap with nitrogen, and desorption of the analytes with an organic solvent or by heating the column. When a solvent is used for analyte desorption, transfer into the GC involved a PTV injection in solvent split mode.

The advantage of OTT over SPE is that the elimination of water is much more efficient, but trapping of the analytes in the stationary phase of the column is not as efficient.

Comprehensive LC×GC

Comprehensive chromatography is a new approach that allows a sample to be separated according to two independent axes. A two-dimensional separation is considered comprehensive if every part of the sample is subjected to two different separations, each with different separation characteristics. Because the separation is performed in two, more or less independent dimensions, the peak capacity is larger, and can be estimated by multiplying the peak capacity of the first- and second-dimension separations.

Most of the applications of comprehensive chromatography involve GC×GC technique. For this technique, instrumentation is also commercially available. Comprehensive chromatography involving other chromatographic techniques (e.g., SFC×SFC, LC×LC, LC×GC) is much less common, but some applications of these comprehensive approaches are starting to appear.

Focusing on LC×GC, from the instrumental point of view, fully on-line automated comprehensive LC×GC is extremely demanding: the GC part must be fast enough to preserve the temporal information of the LC separation. The solvent vapours should be evaporated and rapidly removed in the interface.

Figure 10: Schematic of drop-interface for LC×GC analysis of volatile components. Reprinted by permission of John Wiley and Sons Inc.³²

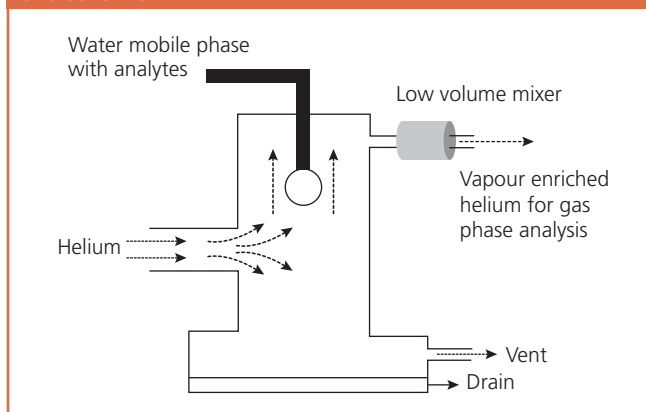


Table 5: Comparison of different evaporation techniques. (Reproduced from ref. 36. Footnote "c" refers to Table 5 of the above mentioned paper). Reprinted by permission of Elsevier.³⁶

Systems	Requirements for LC	Max/min fraction volume (μL)	Elution T of first sharp peaks ($^{\circ}\text{C}$) ^a	Optimization 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, 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	Optimization 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 optimized 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 also suit 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
Vaporizer	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	Optimization demanding

^aFor organic solvents typically used in GC and NPLC–GC.

^bVolatility range from C₆ to C₁₄. ^cSee Table 5

The first system for comprehensive LC×GC has been described in the literature for the analysis of very volatile components (VOCs) in water streams,³² as an alternative to headspace analysis. The interface (termed “drop interface”), shown in Figure 10, consisted of a chamber in which the water mobile phase containing the analytes formed droplets. The VOCs, because of their low to moderate solubility in water, rapidly diffused to the surface of the drop and a substantial fraction was carried away by the helium carrier gas to the GC capillary column for fast GC separation (2 s).

This system is suited only for very volatile components. An LC×GC method has been developed for high molecular mass triglycerides (TAGs).³³ The TAG fraction in edible fats and oils is extremely complex, and to date no single analytical tool exist that has the capacity to separate all individual TAGs present in a matrix. Many methods have been proposed, that involve chromatographic techniques in combination with mass spectrometry, or two-dimensional chromatographic techniques either in heartcutting or comprehensive modes.

The comprehensive two-dimensional LC×GC approach involved the use of a silver-loaded silica column for the LC separation, a PTV interface used in split mode and a short wide-bore GC column for fast GC separation. TAGs were separated accordingly to two independent parameters: carbon number vs number of double bonds. The comprehensive method can be used for fingerprinting of oil samples, as well as for the analysis of target compounds or compound groups.

Concluding Remarks

Coupled LC–GC is an excellent method for sample clean-up and sample enrichment, as demonstrated in the numerous applications published. From its first development, many authors have reviewed in detail the various LC–GC transfer methods and applications.^{10,11,30,34–37}

Table 5 reports a comparison of the different evaporation techniques. For analysis with normal-phase eluents, the main transfer technique is still concurrent eluent evaporation using a loop-type interface. The main disadvantage is that the analysis is restricted to less volatile components. If more volatile components must be analysed, a retention-gap technique must be used. As a result of solvent effects (solvent trapping), the retention-gap technique provides the best retention for more volatile solutes. At the same time, the on-column technique is suitable for the analysis of high-boiling and labile solutes.

Working with reversed-phase eluents, both the loop-type and on-column interfaces presents several disadvantages, and cannot be used routinely. The use of a PTV interface with a suitable adsorbent permits the analysis of more volatile components, but not of high boiling and thermally labile analytes. Direct injection of water via a vaporizer chamber/precolumn solvent split/gas discharge interface can be a good solution for transferring reversed-phase eluents. The drawback of this technique is that only high-boiling solutes can be analysed. Adding a second PTV system before the SVE makes it possible to analyse both volatile and high-boiling components (swing system). The alternative can be the use of indirect methods (phase switching) with LLE, SPE and OTT.

In conclusion, on-line LC–GC is considered a complex technique, and only a few laboratories routinely use it. However, the benefits of on-line coupled LC–GC have been demonstrated: shorter analysis times, better reproducibility and improved detection limits.

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