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Review

High-performance liquid chromatography coupled on-line with high resolution gas chromatography State of the art

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Abstract

Coupled LC–GC is a very powerful two-dimensional system and has potential for substantial time saving in sample separation and sample clean-up. Moreover, efficient elimination of interfering components, better repeatability, and improvement of quantification are possible. This paper provides an overview of the LC–GC transfer techniques developed from its introduction over the last two decades. This review is restricted to direct transfer techniques. © 1999 Elsevier Science B.V. All rights reserved.

Keywords: Liquid chromatography–high resolution gas chromatography; Reviews; Transfer techniques

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1. Introduction

The combination of high-performance liquid chromatography (HPLC) and high resolution gas chromatography (HRGC) in a multidimensional opera-

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tion offers a powerful approach to the solution of very complex separation problems such as the analysis of fuel oils, natural products, and environmental pollutants.

In on-line HPLC–HRGC, the sample is first separated by HPLC using a single column or a combination of columns to isolate the components of interest and then to directly transfer to a capillary column where a further separation is carried out using the high efficiency and sensitivity of HRGC.

The system allows use of the full capability of both LC and GC columns and detectors. This technique is one of the current most powerful analytical tools because of its selectivity and sensitivity in the analysis of complex mixtures. In general, the analysis of very complex matrices is characterised by the complexity of the separation of the components which belong to different classes of compounds and are present in a wide range of concentrations. Many components cannot be resolved in a single GC analysis and the best approach is to fractionate the mixture before the gas chromatographic analysis. The simpler, more homogeneous, mixtures so obtained are easier to resolve without problems of co-elution. Pre-fractionating methods, such as open column liquid chromatography on silica gel or alumina, HPLC, vacuum distillation or preparative gas chromatography have been described. Classical liquid chromatography followed by injection into a GC system is often used for the analysis of trace components in environmental and food samples. LC allows pre-separation and concentration of the components into compound classes and HRGC is used to analyse the fractions, but all off-line techniques are more or less laborious. They entail numerous manual steps (such as extraction or partitioning, transfer from one vessel to another, and evaporation) which introduce the danger of contamination or loss of sample. On-line coupling permits the separation and identification of compounds of the same polarity in mixtures of compounds of different polarity even when the concentrations of each class of compounds are considerably different. Moreover, there is no sample pre- or post-treatment as the separation analysis is fully automated. All these methods have the drawbacks of an off-line pre-separation. Whatever off-line method is employed for solvent evaporation before the injection of the sample into the GC, loss of even high boiling analytes is

likely to happen. In comparison with off-line methods, on-line liquid chromatography–gas chromatography (LC–GC) offers some advantages: the amount of sample required is less and the need to perform laborious sample pre-treatment is greatly reduced.

The introduction of large amounts 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. This review will describe direct transfer techniques for both normal phase and reversed phase eluents. In fact the nature of the eluent greatly influences the choice of the transferring technique as will be explained within the description of each section. All the techniques involving phase switching (e.g. SPE) are not considered in this review.

2. Transfer techniques for LC–GC coupling

In Fig. 1 are summarised the two concepts of eluent evaporation (with some subclasses), allowing transfer of large LC fractions into GC.

2.1. Concurrent eluent evaporation [7–76]

This technique allows extremely precise results and has been described together with the retention gap techniques by Grob [1]. The total eluent evaporation during GC transfer of the eluent allows transfers of fractions of several milliliters. This technique [2] is more satisfactory if carrier gas is used to drive eluent into GC. To prevent the liquid passing the inlet of the GC column, the oven temperature should be slightly above the boiling point of the solvent, corrected for the inlet pressure. In this way the liquid can be transferred at a speed corresponding to the evaporation speed. The loop interface [3], schematically shown in Fig. 2, allows the concurrent eluent evaporation described above, and the liquid is driven by the carrier gas instead of the LC pump.

The fraction to be analysed is contained in a loop placed in a switching valve; the opening of the valve allows the sample contained in the loop to be driven by the carrier gas into the GC. Usually, an early vapour exit, located after a few meters of deactivated

LC-GC ELUENT EVAPORATION TECHNIQUES

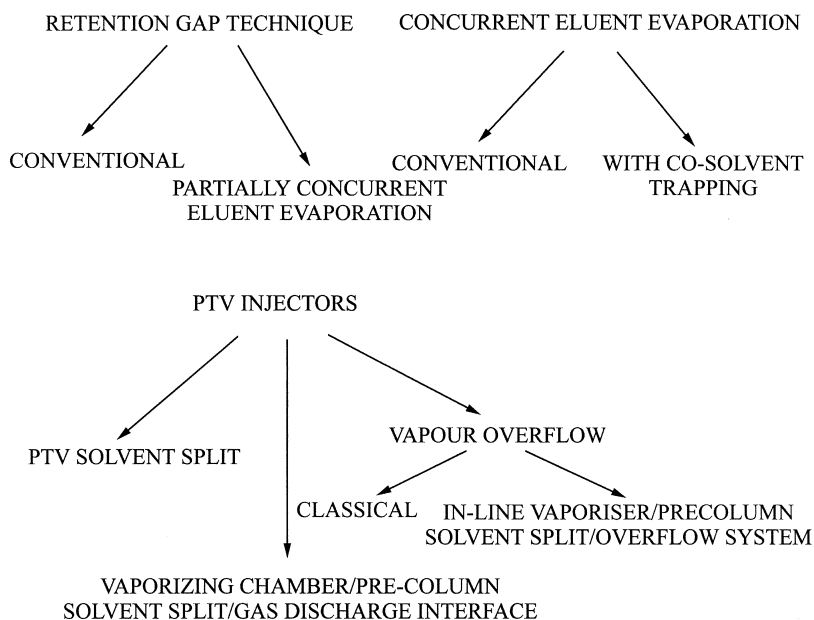


Fig. 1. Description of LC–GC eluent evaporation techniques.

pre-column and 3–4 m piece of retaining pre-column [4] (cut from the separation column) is opened during solvent evaporation to reduce the amount of solvent vapour that goes through the detector and at the same time to increase the solvent evaporation rate [5]. The drawback of the “concurrent eluent evaporation” methods is the loss of low boiling

solutes (see Fig. 3). In fact, as the sample solvent evaporates at the front end of the liquid, no condensed phase remains ahead of the evaporation site. Solutes liberated from the retaining solvent envelope with the solvent immediately start moving into the separation column.

The consequence of solute co-evaporation with the

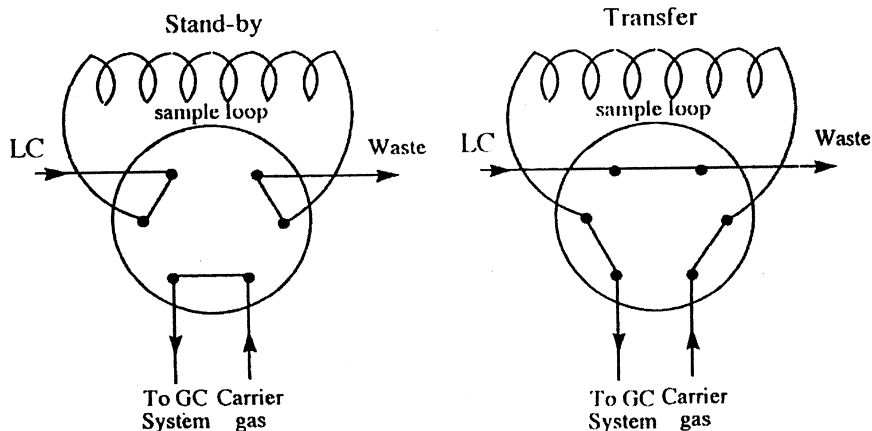


Fig. 2. Loop interface scheme for concurrent eluent evaporation. The sample is first loaded in a loop and then, after the switching of the valve, directed by the carrier into the GC column.

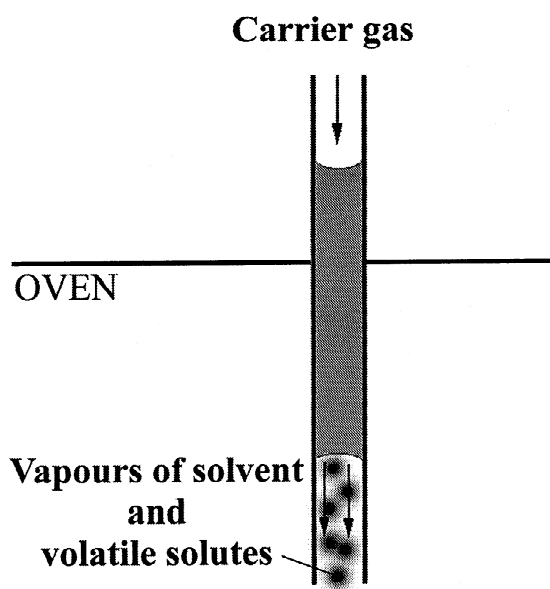


Fig. 3. Concurrent eluent evaporation. The solvent evaporates from the front end of the liquid causing band broadening. Since the column is not flooded very large amounts of liquid can be introduced. Because the eluent evaporates at the front end of the liquid volatile components co-evaporate with the solvent causing loss of sample.

solvent is the loss of volatile solute material resulting either from co-elution with solvent or from venting through the early vapour exit. In practice, the first perfect peaks are eluted between 40 and 120°C above the transfer temperature. This means that concurrent eluent evaporation is only applicable if the elution temperatures of the compounds of interest are above 120–150°C if the eluent is pentane or diethyl ether.

Concurrent solvent evaporation using the loop-type interface is easy to handle, allows transfer of very large volumes of HPLC eluent, and renders solvent evaporation very efficient. In fact, in the literature it is possible to find many applications of this technique. The first reported example [6] demonstrated that with fully concurrent evaporation no flooded zone is produced, and a virtually unlimited volume of liquid can be handled. Up to 10 ml of liquid can be injected in this way at a flow rate of 120 $\mu\text{l}/\text{min}$, requiring an injection time of 83 min.

Fully automated on-line LC–GC methods using the above interface have been developed. In fact, analyses of sterols, waxes, higher aliphatic and triterpenic alcohols, other components of the unsaponifiable fraction of edible oils and fats [7–36],

contaminants of water and food products [37–60], and analysis of fuel products [61–76] have been reported. As an example, Fig. 4 shows the separation by HPLC–HRGC of fatty alcohols, wax esters, free and esterified sterols, as well as minor components for different types of olive oil [12]. As can be seen from Fig. 4, nearly complete absence of wax esters and very low concentrations of steryl esters indicate a high quality extra virgin oil.

As can be seen from Fig. 5, gel permeation chromatography was used for sample pretreatment on a commercially available LC–GC apparatus to separate the low-molecular mass pesticides from the higher molecular mass fat constituents of olive oil [59]. This technique allowed direct analysis of the oil samples after dilution without further sample clean-up. Detection limits were about 5 and 10 $\mu\text{g}/\text{kg}$ with the thermionic and the flame ionisation detector, respectively.

The use of on-line LC–GC coupled to powerful analytical techniques such as AED enables the analysis of complex samples to be simplified. In fact, the presence of trace level heteroatom containing compounds, such as sulphur and nitrogen, can be confirmed by LC–GC–AED, where the unique capabilities of multi-elemental detection are combined. In Fig. 6 is reported the LC–GC–AED analysis of a diesel fuel fraction [75] that represents a powerful analytical approach to PAC elemental information.

2.2. Concurrent eluent evaporation with co-solvent trapping [77–84]

The partial solution to reduce losses of volatile components during concurrent evaporation of the main eluent is to use the auxiliary technique of concurrent eluent evaporation with co-solvent trapping. In fact, the conventional concurrent eluent evaporation suffers from the fact there is no barrier preventing volatile components from co-evaporating with eluent. To overcome this drawback, 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 evaporation site (see Fig. 7), retaining volatile solutes by solvent trapping. The most frequently used co-solvent are alkanes (C_7 – C_{10}) in the case of organic solvents and butoxy-ethanol for aqueous solvents.

Co-solvent trapping has never been used for

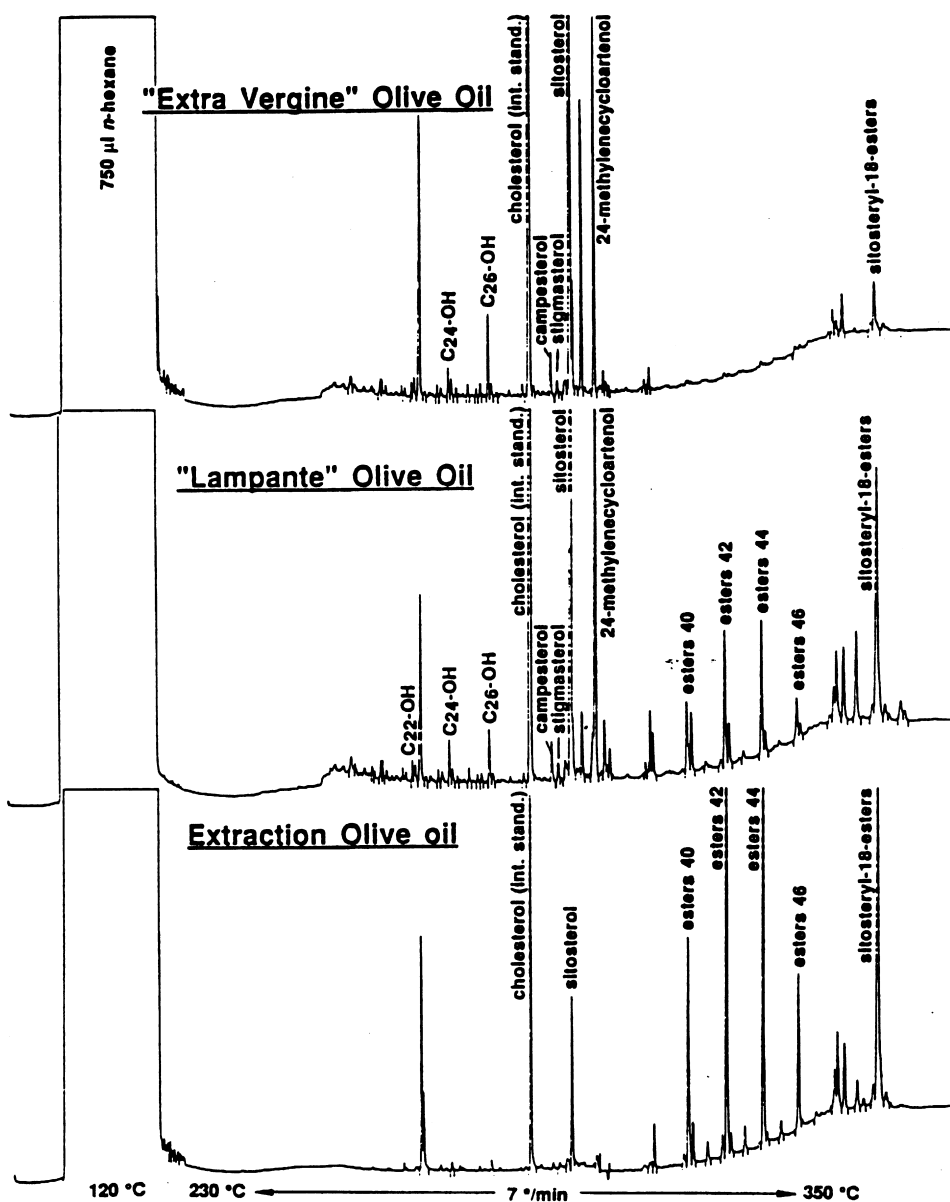


Fig. 4. LC–GC chromatograms of olive oil showing the difference in the insaponifiable composition, between extravirgin, and less valuable “lampante” and “extracted” olive oil. This last chromatogram was obtained after five times sample dilution (from Ref. [12] with permission).

routine analysis of normal-phase eluents because of the superiority of partially concurrent evaporation with the on-column interface for such solvents. Co-solvent trapping was primarily investigated for the transfer of water-containing eluents. The first experimentation with water containing eluent was promising because concurrent eluent evaporation

does not require wettability, and co-solvent trapping retains the volatile solutes, since transfer must occur at 110–120°C and the first solutes could be eluted at these temperatures. However, this technique was not experimented on further, since uncoated pre-columns become adsorptive after few transfers. Fig. 8 [80], for example, shows a chromatogram for a 250 µl

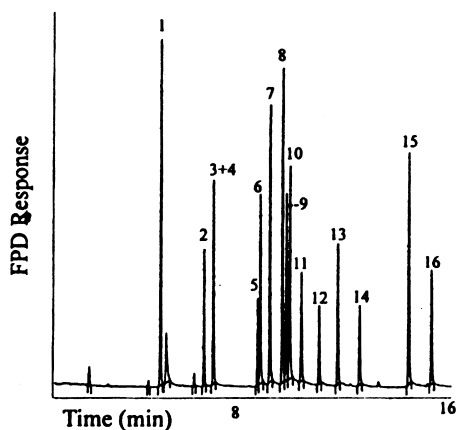


Fig. 5. GPC-GC-FID chromatogram of pesticides pre-separated from high molecular weight components of olive oil (from Ref. [59] with permission).

sample of esters in water containing 22.5% of butoxyethanol as co-solvent. The loss of volatile solute materials is small. Methyl ester peaks down to methyl tetradecanoate (E14) are perfect in shape and size, indicating solvent trapping. Without co-solvent trapping a large amount of E22 is lost. However, water re-deactivates the pre-column surfaces and this is the predominant obstacle to further progress of this technique in reversed-phase LC-GC.

2.3. Retention gap techniques [85–102]

If more volatile components are to be analysed, movement of these compounds into the GC system must be prevented up to the end of eluent evaporation. This is achieved using the retention gap transfer technique, schematically shown in Fig. 9.

The key to this technique is the introduction of the sample at a column temperature below the boiling point of the LC eluent (corrected for the inlet pressure). This causes the sample vapour pressure to be below the carrier gas inlet pressure, and has two consequences: (i) volatile components are reconcentrated by the solvent effects, primarily solvent trapping [1]; and (ii) the high boiling compounds are spread by band broadening in space. A layer of condensed eluent is built up ahead of the evaporation site to act as a thick layer of retaining stationary phase, blocking the further movement of all but the most volatile compounds into the column. Solvent

evaporation, therefore, proceeds from the rear towards the front of the sample layer (see Fig. 10).

However, working with the conventional retention gap technique, due to the limited capacity of uncoated pre-columns to retain liquid, the technique is only suited to the transfer of relative small fractions and involves the use of long uncoated pre-columns. In the literature it is possible to find some applications with the use of the classical retention gap for the transfer from HPLC to HRGC. In particular, Fig. 11 shows the separation of azulene dyestuff in a toothpaste [85]. In this case two fractions were transferred from HPLC; in particular, fraction 1 (150 μl) that contained guajazulene and fraction 2 (270 μl) that contained anethol.

2.4. Partially concurrent eluent evaporation [103–151]

Partially concurrent solvent evaporation allows the transfer of larger fractions than the conventional retention gap technique working under conditions which still produce a zone flooded by eluent (providing solvent trapping) [103]. This technique is a typical retention gap technique, but part of the solvent is evaporated concurrently during its introduction into the GC. The introduction of an early vapour exit [104] improves partially concurrent solvent evaporation. In theory, the solvent vapour exit could be placed between the uncoated pre-column and the analytical column, but this makes the closure critical for partial losses of peaks eluted early. Additional retention power after the solvent evaporation is necessary. A section of the analytical column, a “retaining pre-column” [105] is installed after the uncoated pre-column but before the solvent vapour exit. In Fig. 12 the pre-separation of a neroli essential oil is reported. With the pre-separation of the oil it was possible to transfer two different fractions.

3. Vaporisation with hot injectors

3.1. PTV with solvent trapping in packed beds [152–173]

Cold temperature programmed sample intro-

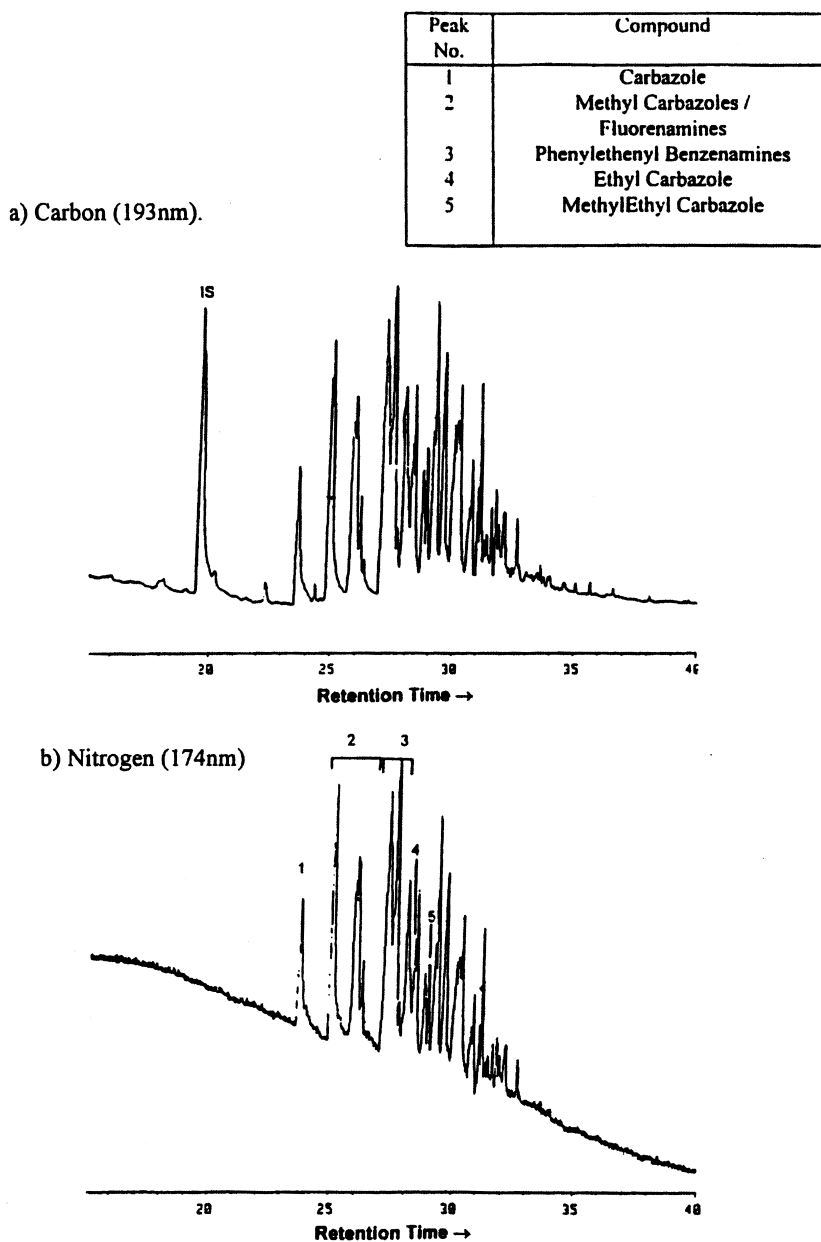


Fig. 6. LC–GC–AED chromatograms of a diesel fuel for PAC determination (from Ref. [75] with permission).

duction is an attractive technique for the introduction of large volumes in capillary gas chromatography and for LC–GC interfacing. The PTV injector has not been largely used because of observed losses of the more volatile components. However, packed vaporising chambers present a lot of advantages

compared to capillary pre-columns. First, wettability is not important for the retention of the liquid and, second, packing materials for the liners are more stable with water. For this reason, introduction of large volumes, mainly of water containing eluents via PTV solvent-split mode has been investigated.

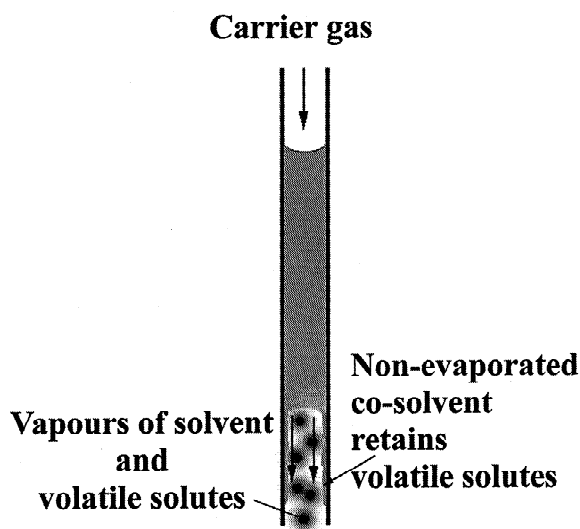


Fig. 7. Concurrent eluent evaporation with co-solvent trapping effect. The high boiling amount of co-solvent creates a layer of condensed liquid where the volatile components are retained.

Due to the large amount of solvent, during the evaporation step the temperature of the injector would decrease. Therefore, higher temperatures are necessary. As a consequence, the main drawback of injection into hot vaporising chambers is when the compounds to be analysed are labile or could give adsorption problems. On the other hand, vaporising chambers better tolerate dirty samples.

Introduction of large volumes in the PTV solvent-

split mode was introduced in the 1970s. In this case solvent evaporation is performed in a glass tube with a packed bed of a cool injector. With the PTV solvent-split injection, the solvent vapours are discharged through the split line. After the end of solvent evaporation the split line is closed before the chamber is heated allowing splitless transfer of the solutes into the column. The group of Staniewski and Cramers [152–154,157,159–160] demonstrated that by reducing the PTV temperature and by increasing the purge gas during solvent elimination it was possible to facilitate solvent removal. Moreover, they used liners with sintered porous glass beads to promote high trapping efficiencies. In fact, solute recovery achieved with this liner was improved compared with liners packed with Tenax TA and ThermoTrap TA. At a liner temperature of -30°C , components with a volatility lower than or equal to that of *n*-tridecane are trapped quantitatively. A different approach was presented by Herraiz et al. [161–163,165]. The peculiarity of the technique described by this research group was given by the transfer of the LC eluent into a packed PTV kept at low temperature. The helium flow rate was established to optimise the solvent removal while the column was detached from the PTV body. Although if this technique presents a bizarre approach (to eliminate aqueous solvents), manually removing the column during an on-line transfer, this method is widely reported in many applications in the literature

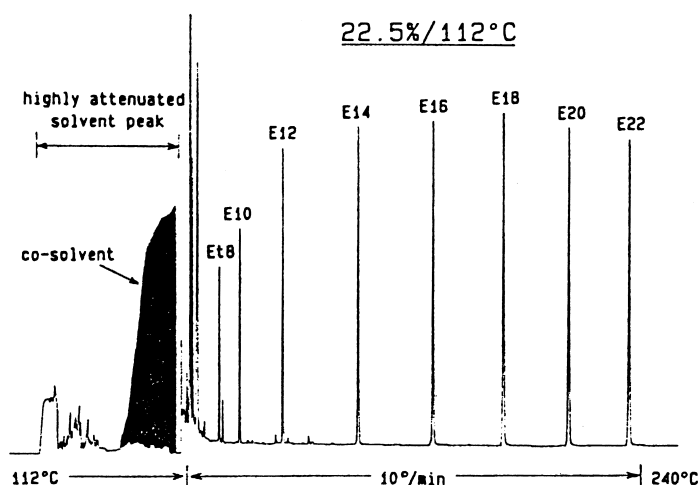


Fig. 8. Methylesters chromatogram, obtained by co-solvent trapping (from Ref. [80] with permission).

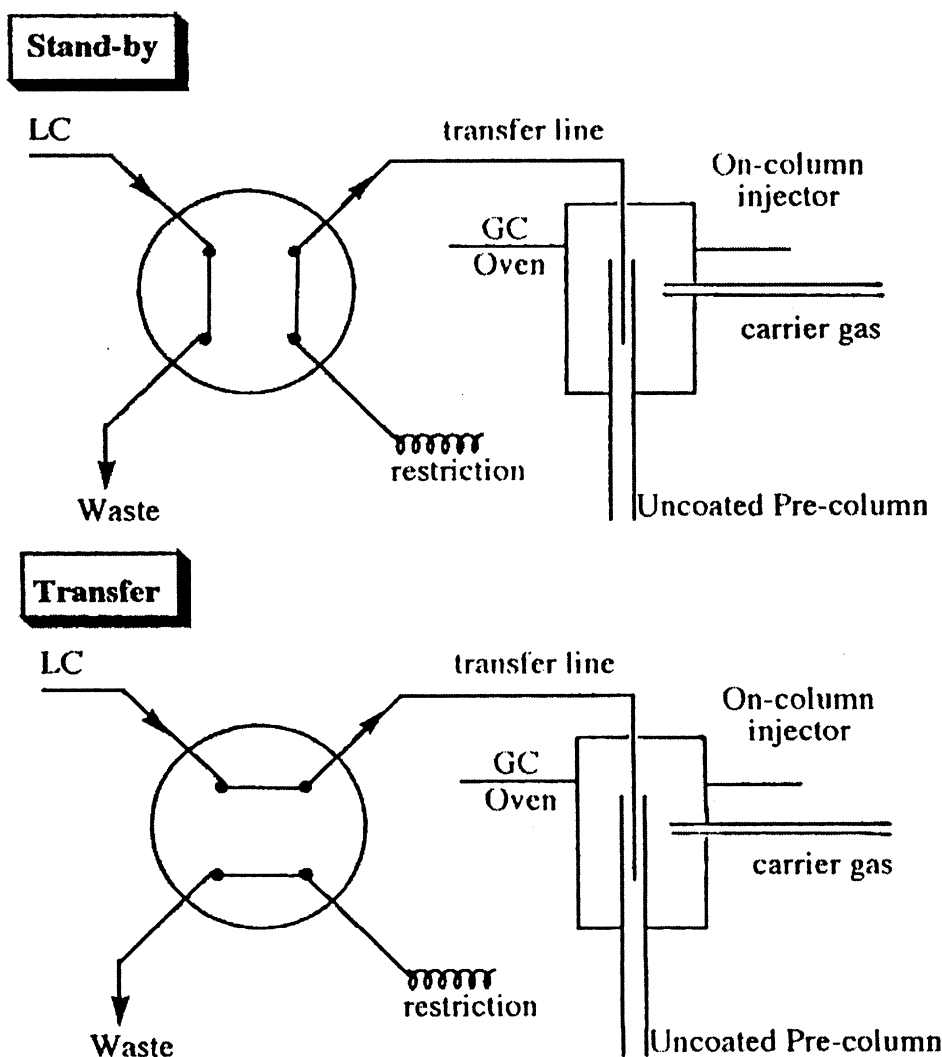


Fig. 9. Retention gap scheme. The eluent from the LC system is pumped through a switching valve to the waste (stand-by position). By switching the valve the eluent with the fraction of interest is transferred via the on-column injector onto the first part of the GC columns (retention gap).

for the determination of sterols, tocopherols and triterpene alcohols and other micro components of edible oils.

Recently, a new, fully automated on-line LC–GC coupling was introduced by Sandra and co-workers [166]. This interface consists of a flow-cell, where the fraction is sampled by a large volume auto-sampler, and automatically injected into a PTV device using the solvent vent mode. This technique was successfully applied for the determination of

pesticides residues in complex natural matrices such as essential oils [169] (see Fig. 13).

3.2. Vapour overflow [174–177]

The vapour overflow technique is intended for introducing samples in large volumes of solvent by syringe injection of strongly diluted samples or by coupled LC–GC. The liquid is introduced into a packed (generally with Tenax) vaporising chamber

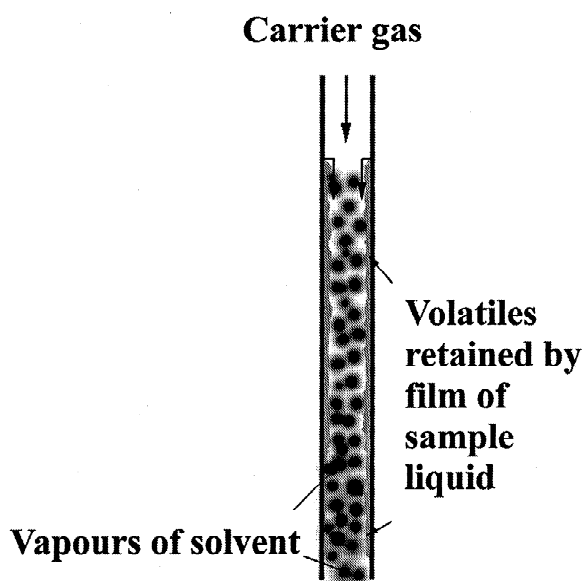


Fig. 10. Solvent evaporation in a retention gap. The liquid from the LC system floods the capillary wall creating a layer that will retain the solutes. The evaporation occurs from the rear part of the solvent refocussing the chromatographic band.

maintained above the solvent boiling point at a pressure which is near or below ambient. This technique is performed in absence of carrier gas and vapours are discharged by expansion during evaporation (overflow). The vaporising chamber is filled with packing material of a gas chromatographic retention power for volatile components. The carrier gas supply line is equipped with a switching valve, allowing the gas supply to be stopped during sample introduction. The sample is released by a syringe or a transfer line from LC near the bottom of the vaporising chamber. Vapours expand and driven by expansion leave the system through the septum purge (see Fig. 14).

After solvent evaporation is completed, the septum purge is closed, the injector is warmed up (PTV) and the trapped analytes are released into the column. More recently, this system has been modified with the use of a conventional hot injector. Fig. 15 shows alkali flame ionisation detection obtained from a solution of some triazines in 1-propanol [175]. This paper demonstrates that the sample volume in split-

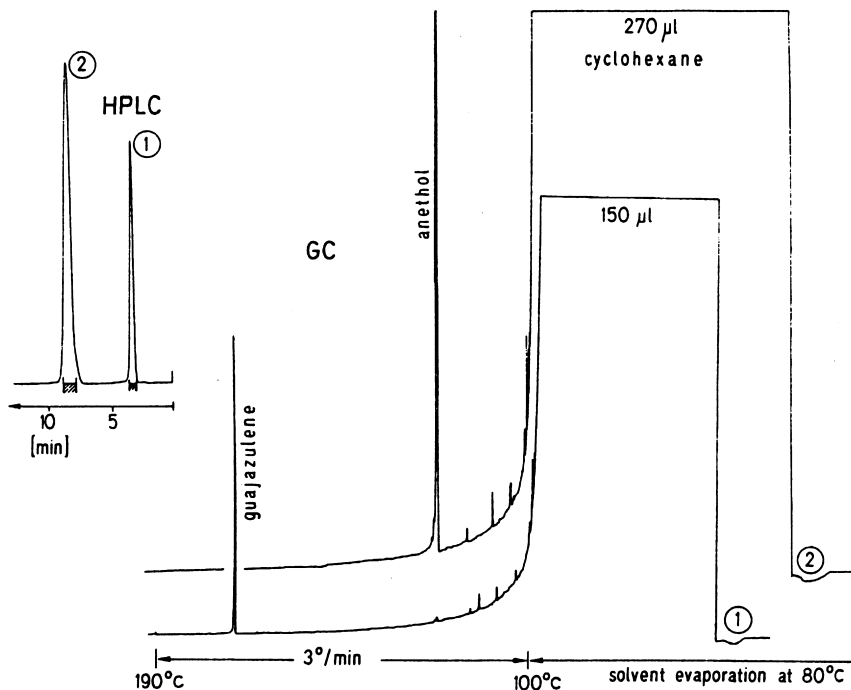


Fig. 11. LC–GC chromatogram using a retention gap interface for the determination of dyestuff in toothpaste. Fraction 1 containing guajazulene; fraction 2 containing anethol (from Ref. [85] with permission).

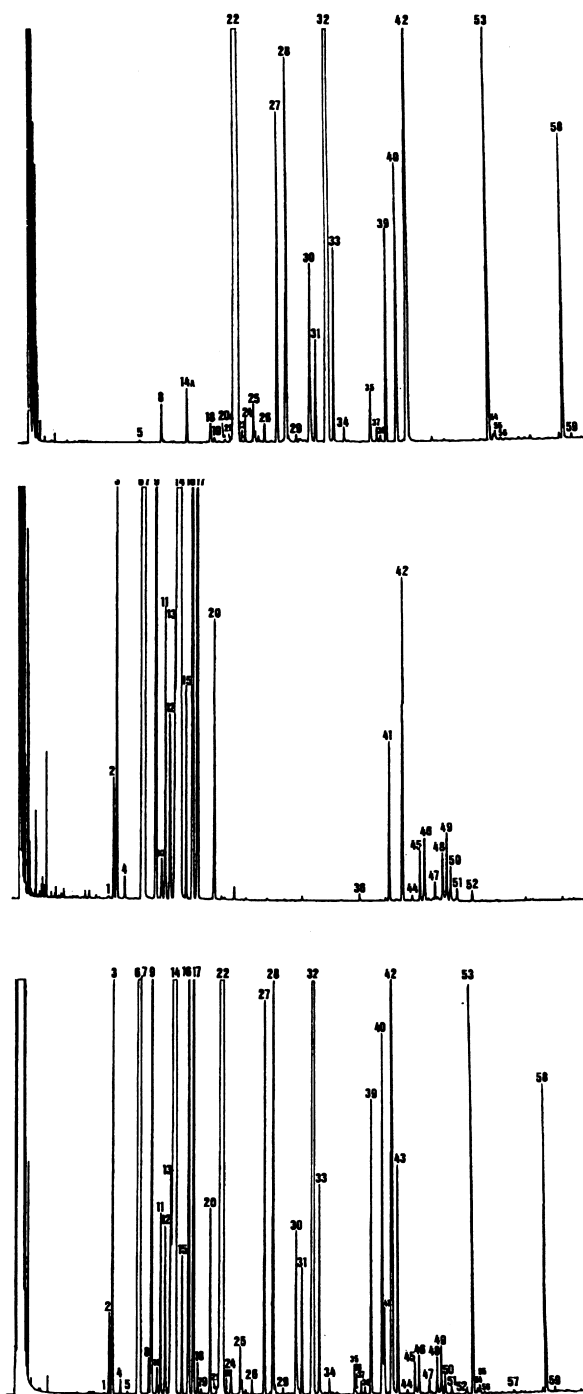


Fig. 12. LC–GC chromatogram of a “neroli2” essential oil. The upper part chromatogram containing the oxygenates while the middle chromatogram containing terpenes were both pre-separated by HPLC. The lower chromatogram refers to the whole oil.

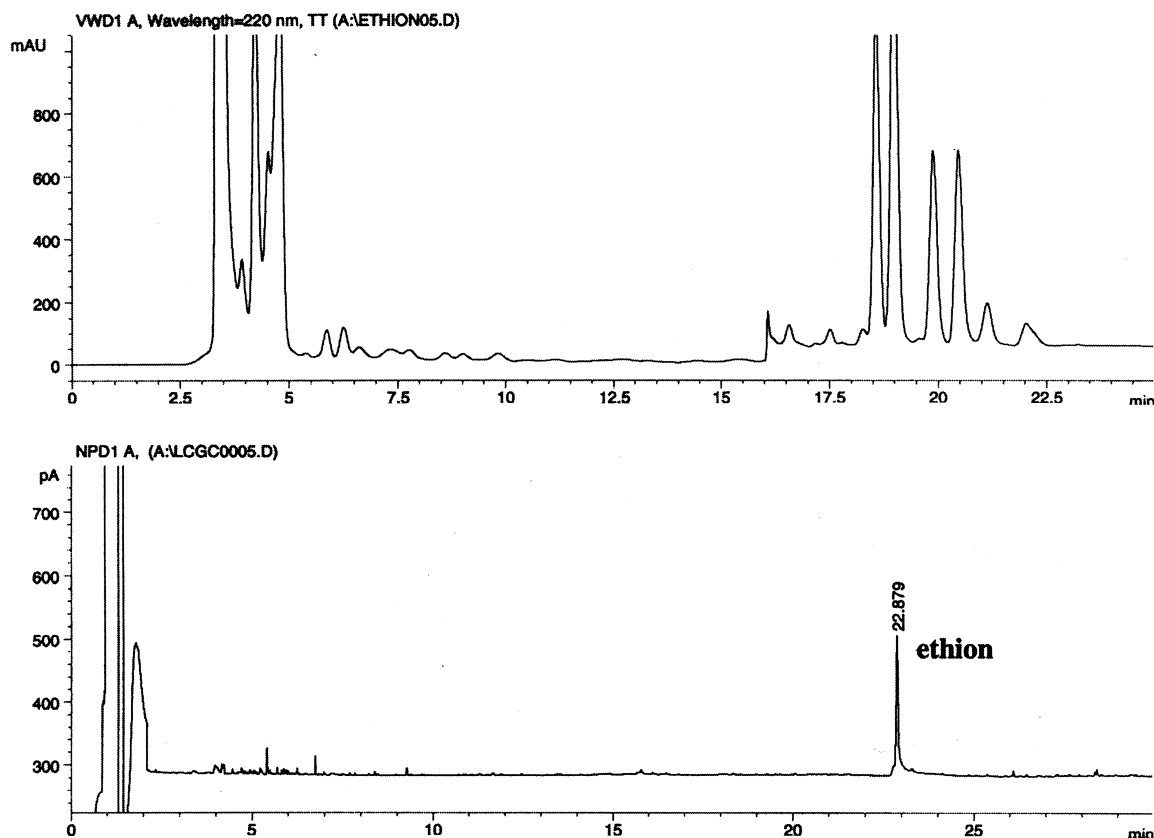


Fig. 13. Upper: HPLC fractionation of orange oil on LiChrosorb 100 Diol. Lower: LC–GC–NPD analysis of orange oil (from Ref. [169] with permission).

less injection can be increased by a factor of at least 100 when the solvent is evaporated with the overflow technique.

3.3. In-line vaporiser/pre-column solvent split/overflow system [177–183]

The in-line vaporiser/pre-column solvent split/overflow system was developed by Grob for large volume liquid injection [178,179] and for on-line LC–GC [180]. This system is an overflow-based technique, but with an improvement to the retention of more volatile compounds. As can be seen from Fig. 16 the vaporiser consisted of a transfer line (from the LC) of fused silica. Inside the vaporiser 5 cm of steel wire (raw or deactivated) or of fused silica was inserted for complete evaporation of the liquid (250–350°C). Moreover, the system is equipped with a retaining pre-column and an early

vapour exit. The oven in this case was maintained at a low temperature (lower than with loop interface) to improve retention of sample components by phase soaking. In fact, as explained in this paper, the improvement corresponds to about four carbon atoms (undecane instead of pentadecane). This system was applied for the analysis of mineral oil paraffins in foods [180], stigmadienols in edible oils [181] and for the determination of mineral oil polyaromatics in foods [182]. Fig. 17, as an example, shows the LC–GC–FID chromatogram of a mineral oil product used as release agent, consisting of isoalkanes [180].

3.4. Vaporising chamber/pre-column solvent split/gas discharge interface [177,184–186]

With this technique the sample is injected by an autosampler or by on-line transfer from an HPLC. The vaporiser is packed and maintained at a tempera-

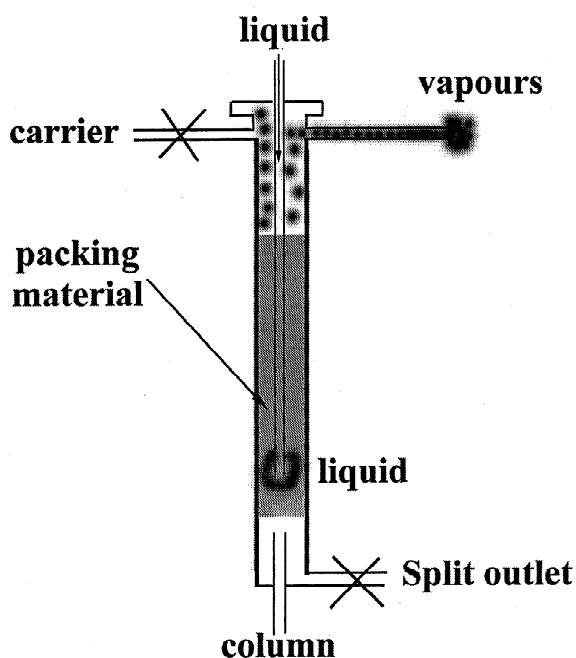


Fig. 14. Vapour overflow interface. The solvent vapours are eliminated through the septum purge of the PTV injector, while the carrier gas is closed.

ture suitable for solute evaporation. For compounds that are sensitive to high temperature it is possible to

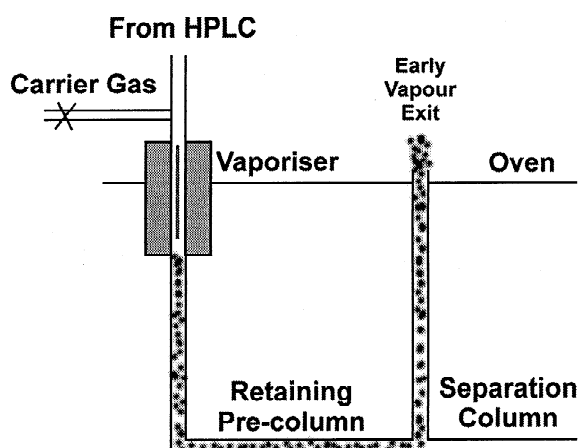


Fig. 16. In-line vaporiser/pre-column solvent split/overflow system. The more volatile components are retained by the retention gap, letting the solvent out from the early vapour exit.

use a PTV injector in order to evaporate the solvent first and then the solutes. As shown in Fig. 18, the vapours are discharged through a pre-column and an early vapour exit. If solvent trapping is needed an uncoated pre-column is installed after the injector and before the solvent vapour exit. Moreover, the use of a retaining pre-column is possible depending on when the solvent vapour exit is closed. For example,

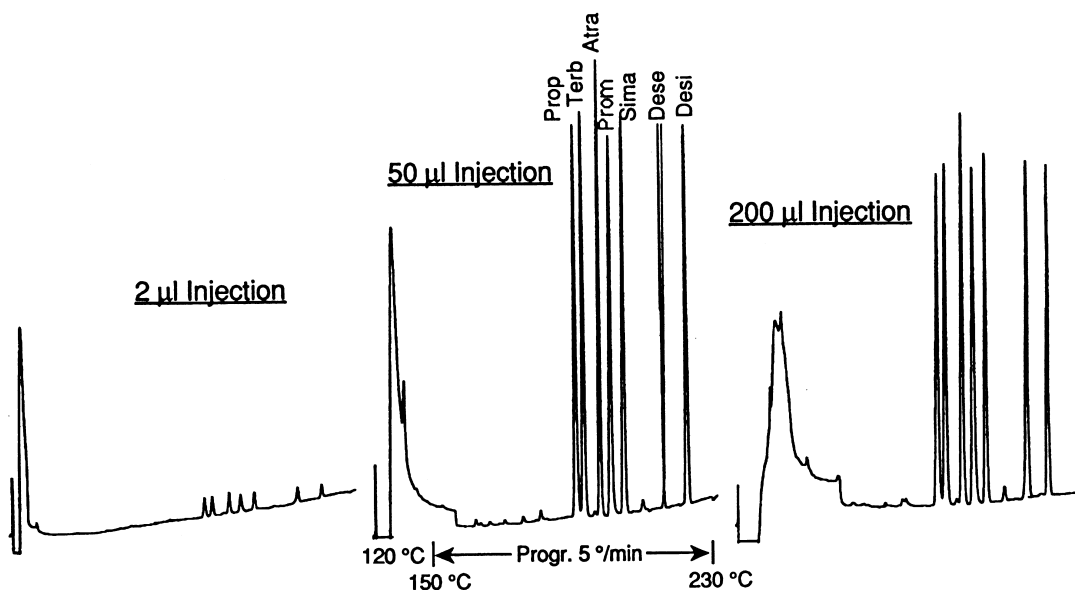


Fig. 15. LC–GC–NPD chromatogram of triazines at very low concentration; the transfer is performed by vapour overflow removal of the solvent (from Ref. [175] with permission).

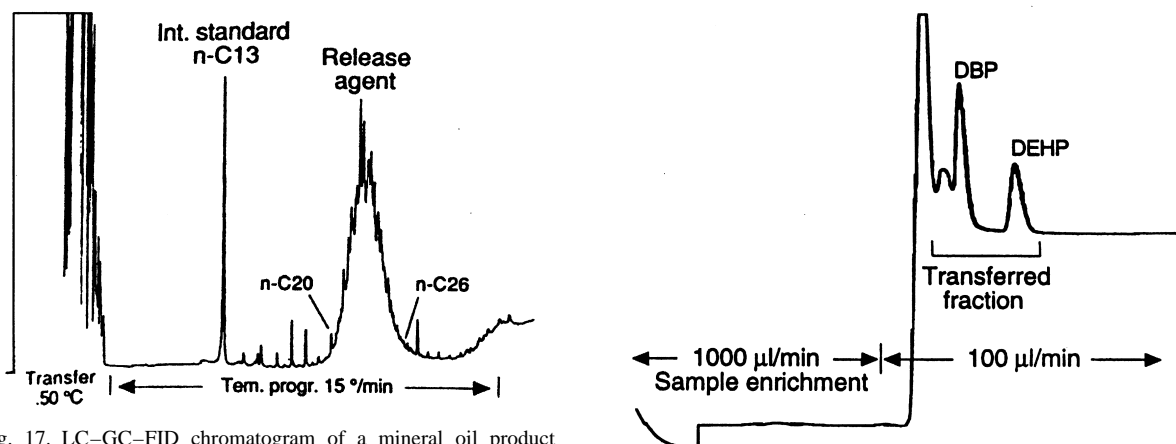


Fig. 17. LC-GC-FID chromatogram of a mineral oil product containing isoalkanes as release agent (from Ref. [180] with permission).

for the determination of pesticides in red wines [186] after reversed-phase sample clean up, the vaporiser/pre-column solvent split/gas discharge interface was used. The eluent (aqueous) is vaporised in a chamber kept at high temperature under high flow-rate. The solvent vapours are removed through the retaining pre-column via a solvent vapour exit, while the analytes of interest are retained on the stationary phase of the pre-column. Fig. 19 shows, in the upper part, a liquid chromatogram of a water sample spiked with some phthalates. In the lower part the LC-GC-MS(EI) chromatogram of a treated drinking water

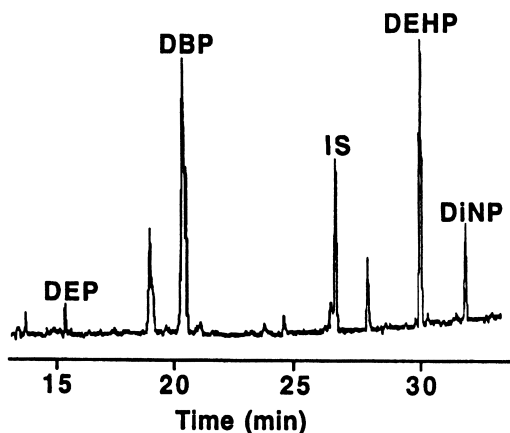


Fig. 19. Above: HPLC chromatogram of a water sample spiked with phthalates (DBP and DEHP). Below: The LC-GC-MS chromatogram of a sample of drinking water (from Ref. [185] with permission).

containing 55 and 40 ng/l respectively of DBP and DHEP is shown [185].

4. Conclusions

Coupled LC-GC has been demonstrated to be an excellent on-line method for sample preparation and sample clean-up. For the analysis of non-aqueous media, the main transfer technique used is, without doubt, concurrent eluent evaporation using a loop type interface. The main drawback of this technique is the solute co-evaporation with the solvent with a

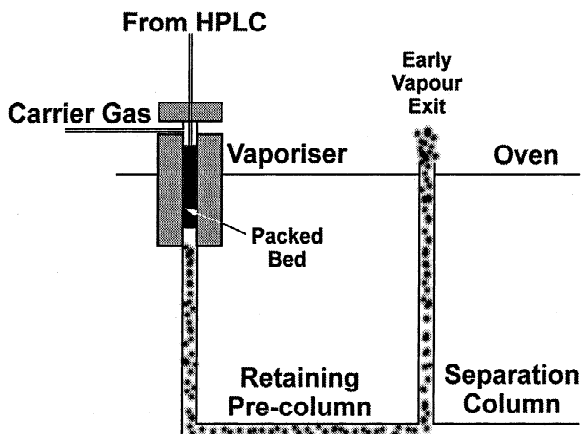


Fig. 18. Vaporising chamber/pre-column solvent split/gas discharge interface. The vaporiser is packed and heated at a suitable temperature for solvent evaporation. The vapour exit can be positioned at the end of the retention gap.

loss of volatile solutes, and the applicability of this interface is severely restricted. If more volatile compounds are involved, transfer from LC to GC is best achieved by retention gap techniques. This on-column method yields sharp peaks starting from the GC oven temperature during eluent transfer. However, due to the limited capacity of uncoated precolumns for liquid retention, the technique is only suited to the transfer of small fractions. Partially concurrent eluent evaporation allows the transfer of larger fractions working under a retention gap procedure. Presently, PTV solvent split injection is considered as the method of choice for large volume injection of “dirty” or water containing samples. Finally, vaporising chamber/pre-column solvent split/gas discharge interface and in-line vaporiser/pre-column solvent split/overflow system seem to be promising techniques for the LC–GC coupling.

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