Laser Light Scattering Detectors

See **II/CHROMATOGRAPHY/Detectors: Laser Light Scattering**

Liquid Chromatography–Gas Chromatography

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Introduction

On-line coupling of high performance liquid chromatography (HPLC) to capillary gas chromatography (GC) means that LC fractions comprising one or several peaks are directly transferred to the gas chromatograph, mostly in a fully automated mode.

The first coupled system, developed by R. Majors in 1980, involved splitless injection by means of a GC autosampler and merely transferred a small part of an LC peak. The first transfer of complete LC fractions was described in 1984. Full transfer is essential, first because of sensitivity (the sample capacity of LC is limited), and second, to obtain reliable quantitative results. The main obstacle to overcome was the introduction of 100–1000 μL volumes of LC eluent into a gas chromatograph.

LC-GC seldom corresponds to ordinary LC with GC added as a detector. Usually GC performs the main analysis and LC is specially designed for a kind of pre-separation or clean-up. The technique presupposes that the compounds to be analysed are amenable to GC, i.e. that they are of limited polarity and rather low molecular mass. For most applications, only normal-phase LC is suitable, either because the matrix material to be injected is not soluble in reversed-phase eluents (e.g. mineral or edible oils), the components are derivatized prior to LC, or the sample consists of an extract from an aqueous phase.

LC columns are kept small (mostly of 2 mm i.d.) in order to keep the fraction volumes below 1000 μL. For the isolation of wider fractions of compounds, solid-phase extraction-type cartridges of lower separation efficiency, yielding smaller fractions, may be more suitable.

In contrast to clean-up by solid-phase extraction (SPE) cartridges, HPLC columns are used over long periods of time. During GC analysis, they are reconditioned, commonly by backflush with a stronger solvent.

Purposes of Coupling LC to GC

Clean-Up

Much of LC-GC serves for the routine analysis of a single or a small group of trace components, i.e. for automated clean-up at high separation efficiency. The LC detector installed between the LC and the GC enables careful optimization of the LC pre-separation and accurate cuts of the window transferred to the GC. Optional 'peak detection' automatically compensates for shifts in LC retention times by the use of the up- and/or down-slope of a peak determined by the LC detector.

Often LC-GC is used for the elimination of time-consuming manual sample preparation. In other instances, only the separation efficiency of HPLC is adequate for the removal of disturbing material of similar characteristics. The determination of traces of ergosterol or Δ8(14)-stigmastenol in edible oils, fats, or food extracts in the presence of far larger amounts of phytosterols are examples. A number of applications even involve two-dimensional LC with heart cutting or LC-GC with intermediate solvent evaporation.

Group-Type Separation

Characterization of complex mixtures often necessitates prior separation into classes of compounds. Examples are the LC fractionation of mineral oil and its products into aliphatics and aromatics of a given number of aromatic rings, the analysis of the pattern of the alkyldibenzothiophenes in mineral oil, the determination of sterol dehydration products for the detection of adulterated olive oils, or the determination of irradiation in fatty foods through olefins cleaved from the triacylglycerols.

Sample Enrichment

Large amounts of some samples can be injected into the LC under conditions that remove the matrix and enrich the components of interest. At least 10 mL of water can be injected, reconcentrating the organic material on, for example, a reversed-phase C_{18} column. Salts are removed and the organic material fractionated by a suitable mobile phase before transfer to the GC.

Transfer of LC Fractions

On-line transfer must be capable of introducing many hundreds of microlitres of LC eluent into the GC. While this has been routine for almost a decade for normal-phase LC eluents (typically based on pentane or hexane), transfer of water-containing eluents is still at an experimental stage, both as a result of technical difficulties, and because of limited applicability.

Routine transfer in on-line LC-GC is mostly achieved by on-column techniques. Usually an early vapour exit is used, releasing the solvent vapours through an outlet installed after a pre-column system. This protects the GC detector and accelerates the discharge of the large volume of vapours (increases the evaporation rate). An uncoated and/or a retaining pre-column is used. The uncoated pre-column serves to evaporate the eluent and reconcentrate the initial bands of higher boiling solutes by the retention gap effect. The coated pre-column retains the solutes during release of the solvent.

Distinction must be made between fully and partially concurrent solvent evaporation. Fully concurrent evaporation means that all the eluent is evaporated during introduction into the GC, i.e. no liquid accumulates in the pre-column system. Volumes of up to several millilitres can be transferred, but volatile components are lost. Partially concurrent evaporation leaves behind unevaporated solvent that must be retained by a relatively long uncoated pre-column and vaporized after the end of the transfer. It is used when solvent trapping is needed for the retention of the volatile components during release of the solvent vapours; components as volatile as heptane can be analysed quantitatively in pentane.

The most obvious alternative to on-column transfer, programmed temperature vaporizing (PTV) solvent splitting, has been proposed, but not described as a routine technique so far.

On-Column Interface

The on-column interface is used for the transfer by partially concurrent evaporation (see **Figure 1**).

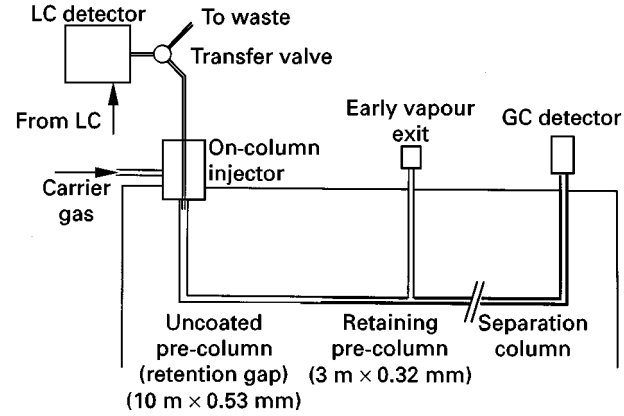

Figure 1 On-column interface for samples containing highly volatile solutes.

Transfer occurs by the same principles as on-column injection of large volumes (retention gap technique). The eluent from the LC passes through a valve for selecting the fraction to be introduced into the GC. It then enters an uncoated pre-column of typically 10 m × 0.53 mm i.d., with a capacity of retaining 100–250 μL of wetting liquid. Partially concurrent evaporation ensures that LC fractions with larger volumes do not overfill the uncoated pre-column. Mostly a retaining pre-column (2–3 m × 0.32 mm i.d.) has been used, but if the early vapour exit is closed before the end of solvent evaporation, it is not really needed. The technique has been routinely used for fractions of up to 800 μL volume.

Loop-Type Interface

Transfer through the loop-type interface (**Figure 2**) is used for fully concurrent evaporation and is suitable for the analysis of components eluted at oven temperatures above about 150°C. It is the technique most frequently used because of its simplicity: the introduction rate is self-adjusting and the end of the transfer

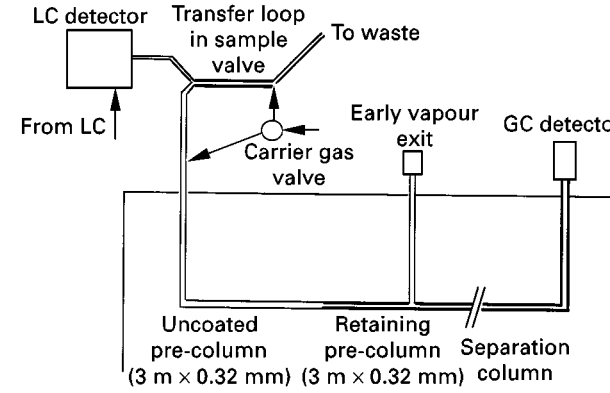

Figure 2 Loop-type interface for transfer by concurrent eluent evaporation.

can be detected automatically. The maximum volume transferred to date is 20 mL.

The LC eluent passes through a loop mounted in the sample valve with an internal volume chosen to match the volume of the LC window to be transferred. At the end of the fraction, as observed by the LC detector, the valve is switched and the carrier gas pushes the liquid from the loop into the GC pre-column. The temperature of the GC oven is above the solvent boiling point at the carrier gas pressure, which causes the liquid to be stopped as soon as it enters the oven-thermostatted pre-column. The vapours are discharged through the coated pre-column retaining the solutes, driven by the pressure of the carrier gas behind the plug of liquid to be transferred (overflow technique). A separate valve actuated simultaneously with the sample valve feeds the carrier gas either behind the liquid to be transferred or to a T-piece allowing purging of the sample valve during analysis.

Stop-Flow Introduction

In 1985, Cortes described LC-GC by a 'stop–flow' transfer technique. The interface used is shown in **Figure 3**. A valve either conducts the LC eluent to waste and supplies the carrier gas to the GC column, or transfers the LC fraction to the GC while the carrier gas flow is interrupted. The vapours are discharged by expansion during evaporation, driven by their own vapour pressure (also called 'overflow').

Vaporizer/Overflow Interface

The vaporizer/overflow interface (**Figure 4**) is a further development of the stop–flow technique. The transfer valve sends the LC eluent either to waste or into a vaporizer chamber typically thermostatted at around 300°C, replacing the uncoated pre-column. Actuated simultaneously, a separate valve (to avoid contact with solvent) stops the carrier gas supply during transfer. The vaporizer consists of 0.32 mm

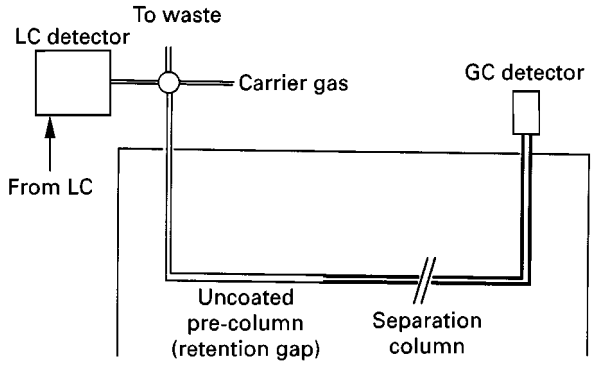

Figure 3 Stop-flow interface.

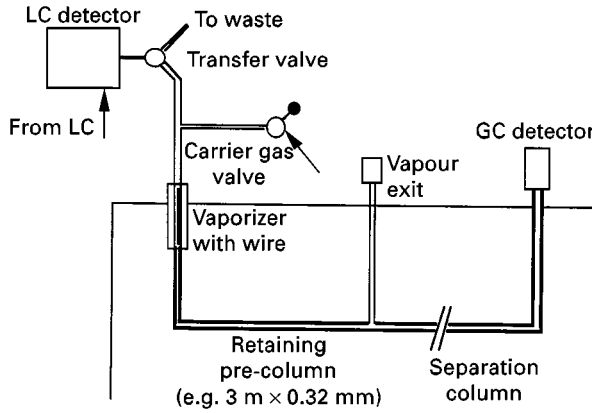

Figure 4 Vaporizer/overflow interface.

i.d. fused silica capillary with a piece of wire (bare metal or metal deactivated by the Silcosteel™ procedure) inserted to prevent liquid from shooting through the chamber. The oven temperature is selected to be near the minimum that avoids solvent recondensation, i.e. at, or slightly above, the boiling point at the pressure required to discharge the vapours (usually 5–15°C above the standard boiling point). A retaining pre-column (typically 2 m × 0.53 mm i.d.) connects to the T-piece of the vapour outlet. Compared with the loop-type interface, the technique improves the retention of the volatile solutes. However, no solvent trapping can be achieved.

Vaporizing Chamber Interface

For the transfer of water-containing eluents or LC fractions containing amounts of nonevaporating material disturbing on-column introduction, a vaporizing chamber is inserted between the on-column injector and the pre-column system (**Figure 5**). It consists of a packed liner of 1–2 mm i.d. and is thermostatted at a high temperature (around 300°C) in order to supply the large amount of heat consumed by solvent evaporation. Vapours are discharged by a carrier gas stream through the vapour exit. An uncoated pre-column is used for partial solvent recondensation if solvent trapping is required, but is of no utility for nonwetting water-containing eluents.

Applications

It is estimated that currently about 200 automated on-line LC-GC instruments are in use. More than half of the applications are in three fields. These were determined by the people involved rather than by particular suitability of the technique and should, therefore, not be understood as an indication that other applications would not be at least equally promising.

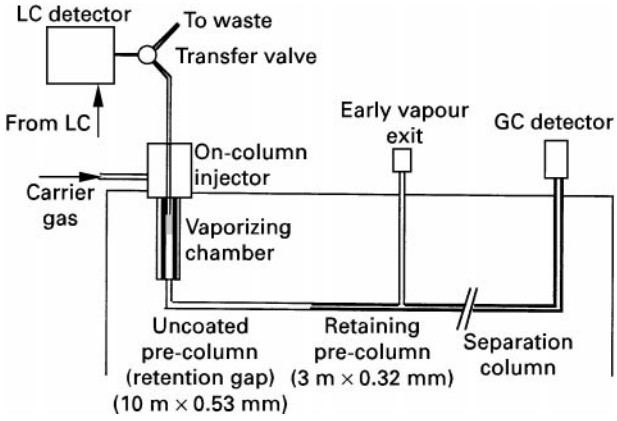

Figure 5 On-column interface with vaporizing chamber.

Mineral Oil Analysis

The petroleum industry has used LC-GC for the pre-separation of products into paraffins and aromatics, as well as for the separation of the highly alkylated aromatics into classes of given ring number (using an amino column). Bartle used the same technique for analysing exhausts from diesel engines. Mineral oil products and their aromatic components have also been determined in foods.

Edible oil

Methods have been developed for the analysis of edible oils or fatty foods in order to achieve faster analysis (circumventing manual clean-up) and analyses of trace components that are difficult to analyse otherwise. They include the analysis of sterols (after transesterification of the oil), the minor components in the oil (after silylation), sterol dehydration products, volatile terpenes in cold-pressed oils, contamination by mineral oil or polyaromatic hydrocarbons, and organophosphorus insecticides.

Figure 6 demonstrates the extremely high resolution achievable by on-line LC-LC-GC-FID for the

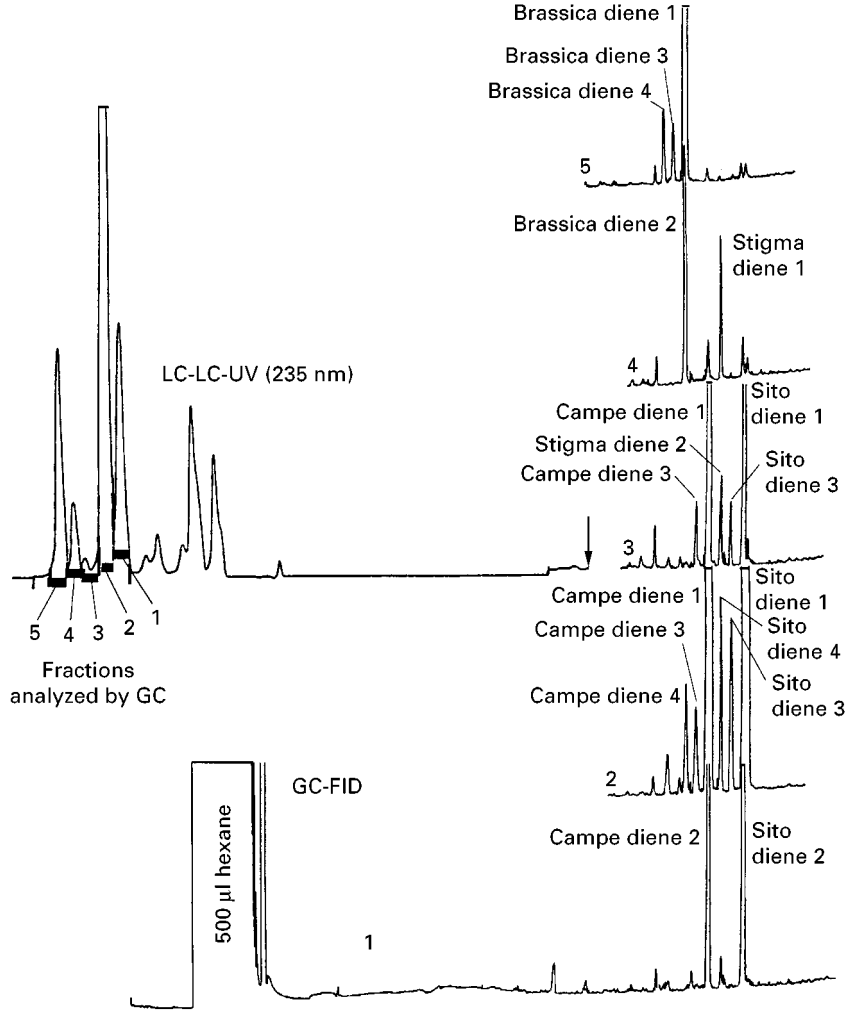

Figure 6 Liquid chromatogram and LC-LC-GC-FID chromatograms of the fractions marked: analysis of sterol dehydration products of rapeseed oil. (Reproduced with permission from Grob K, Biedermann M and Mariani C (1994) LC, GC and GC-MS of sterol dehydration products. *Riv. Ital. Sostanze Grasse* 71: 533–538.

analysis of sterol dehydration products from refined rapeseed oil (the composition of which is of interest for detecting adulteration of other oils, e.g. olive oil). Sample preparation consisted of preparing a 1 : 5 dilution of the oil. The first LC column isolated the hydrocarbons (with the column backflushed by a stronger eluent after each analysis), while the second one separated the products of interest into groups, such that the closely related compounds could be separated by GC. The fractions from LC-LC and the related gas chromatograms are numbered.

Water Analysis

Brinkman, Vreuls, Noij and others have worked on the enrichment of organic materials from water on LC cartridges, followed by on-line liquid or thermal desorption into a gas chromatograph. The aim is a permanent, fully automated analysis of pesticides and other critical contaminants in rivers or the supply lines of water works. A standard procedure consists in extraction of 1–10 mL of water on short polymer-packed LC columns, which are then washed with clean water and dried by a stream of nitrogen. After desorption with ethyl acetate, the sample is transferred through the on-column or loop-type interface.

Conclusion

On-line LC-GC techniques are extremely powerful with regard to selectivity, sensitivity (as a result of the excellent clean-up) and efficiency (as most manual sample preparation is integrated into the automated analysis). It seems, however, that currently they are too demanding for widespread routine use.

See also: **III/Crude Oil: Liquid Chromatography. Terpenoids: Liquid Chromatography. Essential Oils:** Gas Chromatography. **Oils, Fats and Waxes: Supercritical Fluid Chromatography. Petroleum Products:** Liquid Chromatography. **Pesticides:** Gas Chromatography.

Further Reading

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Paper Chromatography

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Introduction

The techniques of paper chromatography and paper electrophoresis are sufficiently intertwined as to be worth considering together, as indeed they often were in books and reviews at the height of their popularity.

Paper Chromatography

The origins of paper chromatography have been traced back by some authorities as far as Pliny (23–79 AD), who described the use of papyrus impregnated with an extract of gall nuts to detect ferrous sulfate. Further examples of the use of paper chromatography can be seen in the 19th-century work of the German chemist Runge who described in his book *Zur Farbenchemie* the use of this type of separation for the investigation of inorganic mixtures. Subsequently another book (*Der Bildungstrieb der Stoffe*) by