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COUPLING OF HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY WITH CAPILLARY GAS CHROMATOGRAPHY

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SUMMARY

A technique allowing the direct transfer of high-performance liquid chromatographic (HPLC) fractions (peaks) on to capillary gas chromatographic (GC) columns is described. Several hundred microlitres of HPLC effluent are pumped through an on-column injector into a retention gap of length *e.g.* 50 m, coupled to a shorter separation column. Reconcentration by solvent effects ensure sharp peaks in isothermal runs. The band broadening in space, visible in runs involving an increase in the column temperature, is eliminated by the retention gap. The analysis of two compounds in a toothpaste by LC–GC and LC–GC–mass spectrometry is shown as an example.

INTRODUCTION

It has long been an aim to couple high-performance liquid chromatography (HPLC) and capillary gas chromatography (GC) because the combination of these fundamentally different separation techniques provides the most efficient two-dimensional chromatography. Clean-up and pre-separation by liquid chromatography (LC) is the most commonly used technique of sample preparation. However, the separation efficiency is poor and the procedure (including calibration and re-checking) is very labour intensive. In many laboratories HPLC is used to pick out a small fraction of a sample with the solute(s) of interest. The solute material is recovered by keeping a vial at the exit of the HPLC detector for the correct period of time. The eluent is evaporated and the sample collected a volume of *ca.* 20–50 μl. The sample volume is often further reduced by a second evaporation step, before 1–3 μl of it are analysed by GC. The method is inconvenient, a large proportion of the sample is lost for the GC analysis and solutes with low to intermediate boiling points are at least partially evaporated during the concentration steps. It is obvious that on-line coupling of HPLC and GC would be most desirable.

Apffel and McNair[1] used an ordinary GC autosampler as an interface between an HPLC detector and the gas chromatograph. The effluent from the HPLC system flows through the syringe of the autosampler to waste. The passing liquid may be analysed by the injection of a few microlitres into a vaporizing injector. This ar-

rangement was used successfully for hydrocarbon group-type analysis with large amounts of sample material, such that sensitivity was not a limiting factor. The technique certainly has a number of interesting applications, but is not LC–GC coupling as desired.

The major problem in the direct transfer of an HPLC fraction into a GC capillary is the volume of liquid involved. A solute eluted from a standard 2–3 mm HPLC column is diluted in at least 150–400 μl of eluent. The sample volumes commonly injected in GC are at least 100 times smaller. During the last 2 years we have developed the basis of a technique that we believe will allow the introduction of several hundred microlitres of liquid. Although many aspects require a more precise treatment, we believe we now have the corner stones of the method.

In isothermal runs at the column temperature during the injection, the solvent effects are the key factor[2]. The sample is introduced for up to 1 min, and the solvent evaporates in the flooded inlet for between 10 min and more than 1 h (depending on the carrier gas flow-rate and the column temperature). Nevertheless, peaks with a width of less than 1 sec are obtained just after the solvent peak. The solvent peak is extremely broad, but it does not obscure many peaks, because the retention times of the latter are increased accordingly. However, full solvent trapping and/or strong phase soaking is required. Partially trapped solutes which are not reconcentrated by phase soaking elute as distorted peaks with a width of up to 1 h.

If the elution temperature of the solutes of interest is above the injection temperature, the band broadening in space is the dominating factor[3]. The liquid introduced floods a column length of 30–100 m and the solutes are spread accordingly to give an extremely broad band. These bands may be reconcentrated by the use of a retention gap[4], using an uncoated pre-column with a length that corresponds at least to that of the flooded zone. The solute bands are reconcentrated by the ratio of the retention powers in the retention gap and the separation column[5]. A 50-m retention gap may be connected to a 15-m separation column and the separation power of the system is not noticeably reduced compared with that of the separation column alone, whether used for the introduction of small or large sample volumes. Peak broadening due to longitudinal diffusion is negligible under the usual conditions[6].

EXPERIMENTAL AND RESULTS

Equipment

HPLC. A 100 × 3 mm I.D. glass column, packed with Spherisorb S-5W, an injector (Waters U6K) and a UV detector (Kratos, Spectroflow 773) operated at 274 nm, 0.4 a.u.f.s., were used. The eluent was cyclohexane, distilled over $LiAlH_4$, 45 bar, flow-rate 400 μl/min.

GC. Standard equipment with an on-column injector (Carlo Erba) was used. A fused-silica retention gap, 50 m × 0.32 mm I.D., leached and persilylated with diphenyltetramethyldisilazane (from MEGA through Carlo Erba), was coupled with a butt connector (Carlo Erba) to a 30 m × 0.30 mm I.D. glass capillary coated with 0.6 μm SE-54. The inlet pressure (hydrogen) was 1.7 atm.

Interface. The arrangement of the equipment is shown in Fig. 1 and details of the interface in Fig. 2. The sample was introduced into the retention gap through a 0.17 mm O.D. fused-silica capillary (also used for the preparation of on-column

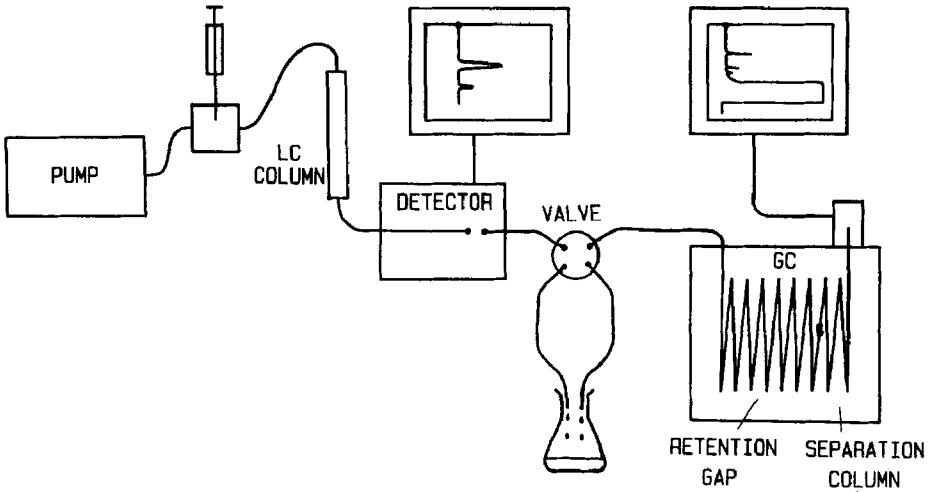

Fig. 1. LC–GC arrangement with the HPLC detector linked to a switching valve, leading the effluents from the HPLC column into the retention gap (uncoated pre-column) in the GC system or to waste.

syringe needles) passing through the on-column injector 6 cm into the retention gap. To avoid permanent leakage of the injector, the fused-silica capillary was drawn through a septum which was pressed on to the injector using the stand commonly used as a guide for syringe positioning.

The centre of the interface was a four-port switching valve (Valco, VICI, Schenkon, Switzerland). In the stand-by position the HPLC eluent flowed to waste. At the same time, the fused-silica capillary between the valve and the retention gap

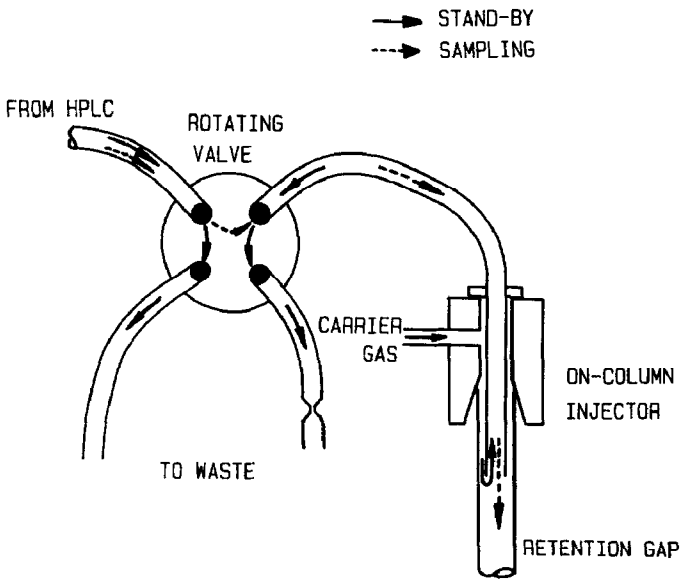

Fig. 2. Detail of the LC–GC coupling. In the stand-by mode (with the HPLC or GC systems running on their own) the effluent from the HPLC detector passes through the rotating switching valve to waste. The connection to the GC system is backflushed by the pressure of the carrier gas in the column inlet. In the sampling mode the HPLC pump pushes the effluents into the retention gap in the GC oven.

was purged backwards by a stream of carrier gas, driven by the column inlet pressure through the switching valve and another tube leading to waste. The latter tube contained a restriction adjusted to allow a gas flow-rate of about 5 ml/min at 1 atm inlet pressure in the gas chromatograph. In the sampling position of the switching valve the liquid from the HPLC detector was pushed against the GC inlet pressure into the retention gap by the pressure from the HPLC pump. The dead volume of the interface was 14 μl, resulting in a retardation of the sample material in the GC part of 2 sec (at a liquid flow-rate of 400 μl/min).

LC–GC of fluorene

A 2-μl volume of a 30-ppm solution of fluorene in dichloromethane was injected on-column on to the GC column alone (removing the LC–GC transfer line). The resulting chromatogram (Fig. 3) served as a reference. A 2-μl volume of the same solution was injected on to the HPLC unit and transferred with 170 μl of eluent (cyclohexane) into the capillary column kept at 80°C. The column was kept at this temperature (the boiling point of the solvent at ambient pressure) until the solvent peak had passed the column (14 min), then programmed as indicated in Fig. 3. The peak area of the transferred fluorene corresponded to that of the injection into the GC system, indicating complete transfer. The peak width increased insignificantly from 4.85 to 4.95 sec, demonstrating virtually complete reconcentration of the band broadening in space by the retention gap. The length of the flooded zone was estimated to be 20 m.

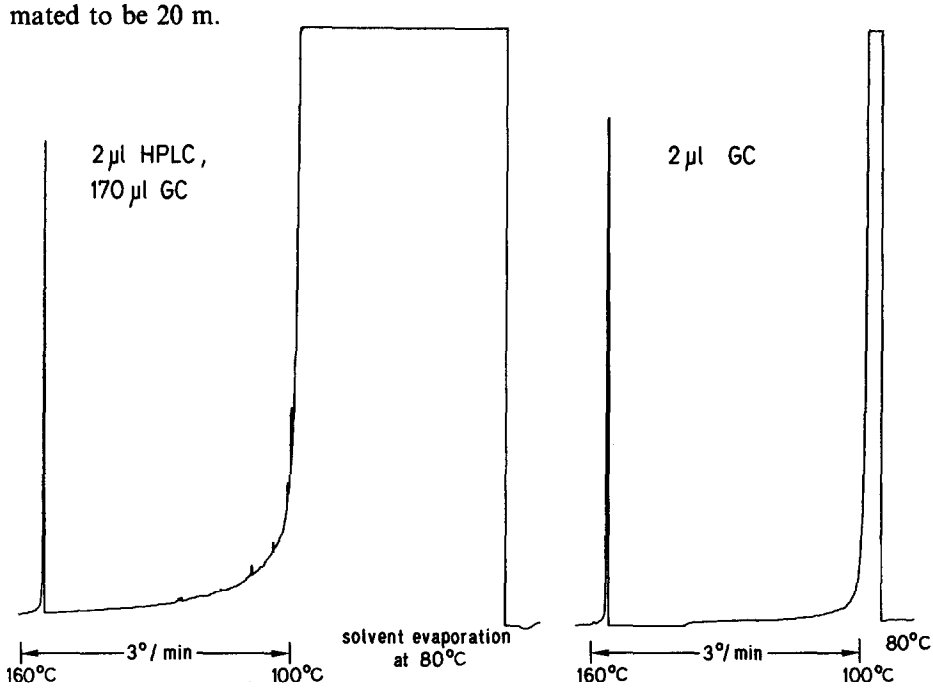

Fig. 3. A 2-μl volume of a 30 ppm solution of fluorene either directly injected into the retention gap in the GC system (right) or on to the HPLC column and transferred with 170 μl of cyclohexane (eluent) into the gas chromatograph. The comparison of the peak areas indicates complete transfer of the solute material. The heights and the widths of the peaks are nearly identical, also showing complete reconcentration of the bands broadened in space.

Dyestuff in toothpaste

A toothpaste had to be analysed because it was claimed to contain azulene as a dyestuff. Using HPLC on silica columns azulene and alkylated azulenes could not be separated. Further, we did not have sufficient reference substituted azulenes to confirm the identification of the sample compounds by co-chromatography using HPLC. Capillary GC separates the azulenes far better than HPLC. Direct injection of the crude and concentrated methanol extract of the toothpaste produced a large number of peaks, but GC detectors were unable to establish which of these peaks correspond to azulene and its substituted products. Further, the crude extract severely contaminated the GC system.

LC–GC solved both problems. The gas chromatogram of the observed LC peak (No. 1 in Fig. 4) contained only one major peak, which was identified as guajazulene (1,4-dimethyl-7-isopropylazulene), and co-chromatography using capillary GC is considered to provide a highly reliable identification in this instance. HPLC removed all the interfering peaks and also the involatile by-products, thus effecting a clean-up and a pre-separation.

The HPLC trace (Fig. 4) contained a second peak, which also gave a GC peak (LC–GC, see Fig. 4). It was identified by mass spectrometry (MS). The sample was

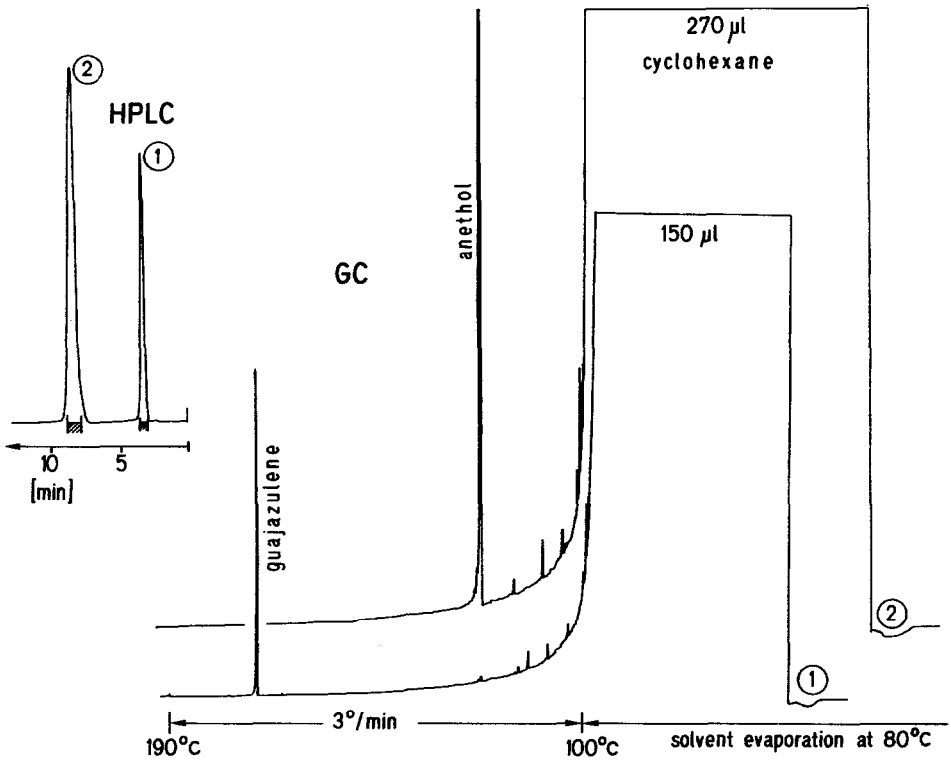

Fig. 4. LC–GC of two solutes visible on the HPLC trace (left),run for the analysis of azulene dyestuffs in a toothpaste. Peak 1 was transferred with 150 µl of eluent and coeluted in the GC system with guajazulene. Peak 2 was identified as anethol by LC–GC–MS.

transferred from the HPLC system on to the GC column and the solvent evaporated (observation with a flame-ionization detector). The column was then transferred into the GC–MS unit and the compound was identified as anethol.

DISCUSSION

We believe that the proposed technique for LC–GC coupling has great potential. In particular, the volumes of liquid transferred, the most critical factor, are still far from the possible limit. However, further investigations of a number of parameters are needed in order to establish firm guidelines for users. These aspects are considered below.

The maximum tolerable length of the flooded zone is usually determined by the band broadening in space and the reconcentration by the retention gap (not important for fully isothermal runs). This length depends on the retention power in the retention gap[5], the film thickness of the stationary phase in the separation column, the separation efficiency of the separation column and the ratio of the inner diameters of the retention gap and the separation column.

The length of the flooded zone for a given volume of liquid is a function of the wettability of the internal wall of the retention gap by the solvent[7], and to some extent also of the carrier gas flow-rate and of the GC oven temperature during the sample introduction. The dependence of the length of the flooded zone on the column temperature was first reported by Trestianu *et al.*[8] and further studied by the same group[9], Wang and co-workers[10,11], Yang[12] and ourselves[7]. An increase in the temperature of the retention gap easily halves the length of the flooded zone or doubles the capacity of a retention gap.

The evaporation of the solvent in the retention gap is time consuming. Even if the carrier gas passing the column were fully replaced by solvent vapour, the evaporation of 400 μl of liquid creates a vapour volume of 100–200 ml, which passes the column in 25–50 min if the flow-rate is 4 ml/min. This explains the interest in a high column temperature (to increase the proportion of sample vapour in the passing carrier gas) and a high carrier gas flow-rate (hydrogen!). The column temperature must not be too high, because otherwise large amounts of liquid may be pushed backwards out of the retention gap through the purge of the LC–GC interface.

The temperature programme during the GC analysis must not exceed a certain rate in order to give the migration of the solutes in the retention gap enough time.

HPLC columns with a small inner diameter dilute the solutes in less eluent. For the same reason, relatively short and highly efficient columns are preferable, but standard 2 or 3 mm I.D. columns appear to be acceptable.

Reversed-phase HPLC creates more problems for LC–GC than adsorption chromatography. The length of the flooded zone for a given solvent volume is increased owing to wettability problems in the retention gap. The addition of salts, acids, bases or ion-pair reagents is excluded. Finally, many early eluted solutes are partially solvent trapped. As very polar solvents seldom create a phase soaking effect, these solutes produce distorted peaks, and the distortion is far more disastrous than for the conventional sample volumes. Despite these restrictions, there should remain an interesting range of applications for reversed-phase HPLC–GC.

CONCLUSIONS

The use of large retention gaps is not the only way to couple HPLC and GC, but it allows the construction of combined equipment with most existing on-column injectors and GC ovens. It provides reliable quantitative results as it involves the same simplicity of sample introduction as on-column injection. Finally, it uses a simple concept, with the advantage that only a few parameters need to be mastered in order to control the method.

We expect the most interesting applications of LC–GC in three fields as follows.

(a) Clean-up and pre-separation for the analysis of trace components in complex mixtures (environmental samples, biological fluids, foods). HPLC is more efficient than classical liquid chromatography and is easily automated. Column switching techniques might be further developed to allow a start with relatively large volumes of crude samples, first cleaned by a large column and then reconcentrated in a narrower bore column coupled to a GC system. The use of size exclusion chromatography is an attractive solution for solving the problems caused by involatile by-products (to which the retention gaps are sensitive). Maybe the dream of fully automated sample preparation will approach reality.

(b) Group separations according to polarity by HPLC, followed by separation according to volatility (GC), which is real two-dimensional chromatography for complex mixtures such as hydrocarbons (paraffins, olefins, aromatics), cigarette smoke (*e.g.*, for the analysis of polynuclear aromatics) or fatty acids (for separating *cis*- and *trans*-mono-unsaturates).

(c) LC–GC–MS. As LC–MS is a method with many problems, the combination with GC is attractive for analyses of solutes that are amenable to GC. The solutes are further separated and at the same time reconcentrated in a small volume of gas, instead of several hundred microlitres of liquid.

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