On-line liquid backflush of an uncoated precolumn for automated gas chromatographic analysis of complex mixtures

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ABSTRACT

A fully automated system for the GC analysis of samples containing non-volatile material, consisting of a double-oven on-line liquid backflush system, was constructed and evaluated. An uncoated precolumn is mounted in the first oven and the analytical column is housed in the second oven. It is possible to backflush the precolumn with both gases and liquids, *via* low-volume three-way connectors, while the analysis is proceeding in the coated column. The different events are controlled pneumatically by off-line valves, situated outside the GC ovens. The performance of the system was evaluated by repetitive injections of sample solutions containing crude oil, olive oil and shoe polish. It is shown that refocusing of high-boiling solutes from contaminated precolumns is enhanced when a faster temperature ramp of the precolumn is utilized. It is also shown that the useful lifetime of the precolumn is significantly extended by the on-line solvent rinsing of the precolumn.

INTRODUCTION

The performance of automated GC analyses of complex mixtures is often gradually impaired owing to the accumulation of non-volatile material in the injector or in the column entrance. Trace analysis, using the retention gap technique, is particularly sensitive in this respect as the uncoated precolumn tolerates only a very limited amount of non-volatile material [1,2]. When this limit is exceeded, extra-column band broadening and/or peak splitting occur.

In order to avoid this problem, an off-line sample clean-up is often employed. Techniques such as liquid–liquid or solid-phase extraction are, however, labour demanding, time consuming and not as reproducible as direct introduction of the sample. In this respect, on-line coupled LC–GC is a better approach [3]. A separation

on the basis of polarity may remove the non-volatile components present in the sample as long as these do not co-elute with the volatile solutes of interest. Size-exclusion chromatography can also be employed when the resolution between the high- and low-molecular-mass fractions is sufficient [4–7]. Problems such as adsorption of sample material on the LC column and contamination of the LC column should, however, be taken into consideration.

Continuous monitoring of trace components, to perform large series of routine laboratory analyses or on-line measurements in industrial processes should produce reliable results during long periods of time, preferably without supervision. At present, capillary GC is not capable of fulfilling these criteria when the sample contains non-volatile compounds. Recently, we presented the principle of a GC technique which could be a remedy for this problem [8]. A double-oven GC system was utilized where an uncoated precolumn was independently temperature pro-

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grammed from the column and subsequently backflushed with a solvent during the analysis. By employing a faster temperature ramp for the precolumn than for the analytical column, the solutes that are spread over the precolumn reach the entrance of the analytical column while it is at a lower temperature than in the traditional retention gap set-up [9–11]. Consequently, a more effective solute focusing effect is obtained while the presence of non-volatile material becomes less critical. An additional liquid backflush of the retention gap removes most of the non-volatile material and restores the system performance between each analysis.

The performance of this technique was demonstrated with a system where the uncoated precolumn and the analytical column were isolated by an in-line valve [8]. In this paper, a computer-controlled, fully automated GC system that utilizes pneumatic (Deans) switching [12–15], which eliminates the need for in-line valves, is presented.

EXPERIMENTAL

Equipment

A schematic diagram of the experimental set-up is shown in Fig. 1. Two gas chromatographs (Varian Model 3700) were utilized with an uncoated precolumn [8 m × 0.32 mm I.D., deactivated with diphenyl-trimethyldisilazane (DPTMDS)] mounted in the first oven and an analytical column [30 m × 0.25 mm I.D., coated with 0.25 μm DB-5 (J&W Scientific)] housed in the second oven. Three-way press-fit connectors were prepared by fusing three borosilicate glass press-fit connectors together, guided by 0.25 mm I.D. bare fused-silica tubes as described elsewhere [16].

V_1–V_6 were Valco rotary valves of the CW and NW series, pneumatically actuated by digital valve interface units. The sample loop and the solvent loops were filled from glass reservoirs pressurized *via* three-way solenoid valves (Model 339; Asco Controls). The system was fully auto-
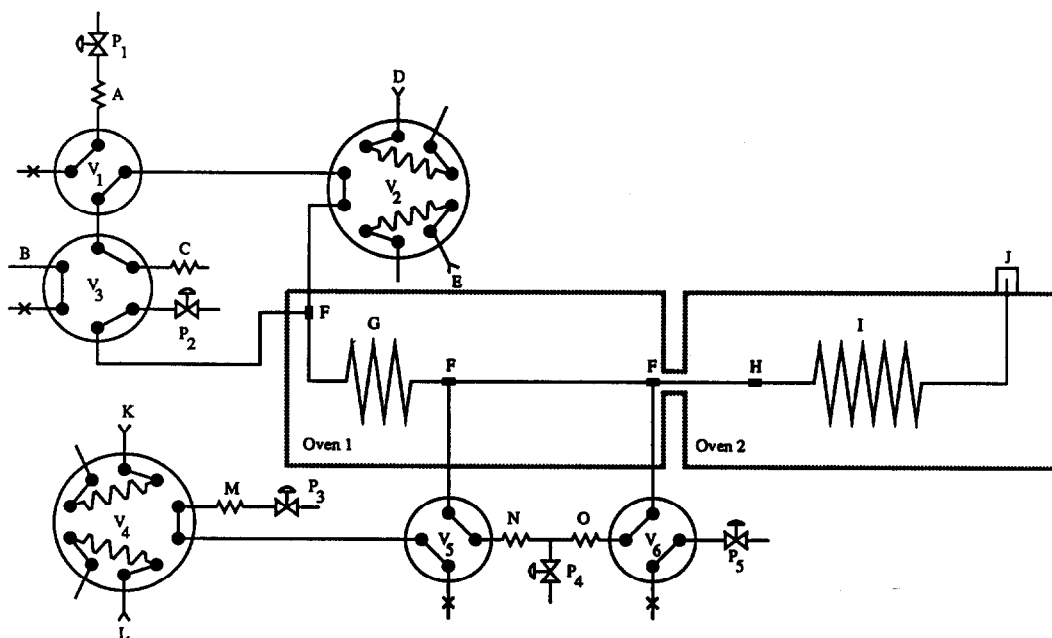

Fig. 1. Schematic diagram of the experimental set-up. A = Restrictor (1 m × 52 μm I.D.); B = waste outlet; C = restrictor (60 cm × 52 μm I.D.); D = wash loop (10 μl) inlet; E = sample loop (10 μl) inlet; F = three-way press-fit connector; G = uncoated precolumn; H = press-fit connector; I = analytical column; J = flame ionization detector; K = backflush solvent loop (500 μl) inlet; L = backflush solvent loop (500 μl) inlet; M = restrictor (60 cm × 52 μm I.D.); N = restrictor (1 m × 52 μm I.D.); O = restrictor (1 m × 52 μm I.D.); P_1–P_5 = pressure regulators; V_1–V_6 = GC switching valves.

mated from a personal computer equipped with a relay driver card (PC-36/A 8255; ELFA) and a electromechanical relay adapter unit (PC-38; ELFA). The different events were time controlled by contact closures of the relays with a computer program written in Pascal.

Pressure regulator P_2 provides the carrier gas pressure during injection and the sample refocusing stage and P_5 provides the carrier gas pressure in the backflush mode. P_1 (and restrictor A) serves to inject the sample at a controlled rate and was set at a higher pressure than P_2. P_3 (and restrictor M) controls the rate of backflush solvent introduction and was set at a higher pressure than P_5. P_4 provides a small purge flow to the three-way press-fit connectors via restrictors N and O to avoid dead volumes in the connector legs and was set at a higher pressure than P_2 and P_5. Restrictor C ensures a backflush purge flow of the cold part of the transfer line from valve V_2 to avoid slow release of residual solvent from the transfer line and the valve after sample transfer.

Procedure

The operating sequence of the system is outlined below.

(1) In the load position (where the valves V_1–V_6 are in the positions shown in Fig. 1) the sample loop, the wash loop and the liquid backflush loops are filled with sample, wash solvent and backflush solvents, respectively.

(2) The sample and the wash solvent are transferred to the uncoated precolumn by switching valves V_1 and V_2 (Fig. 2A).

(3) When the sample transfer is completed, valves V_1 and V_2 are switched back to the initial position. Remaining solvent residues in the cold transfer line are thereby backflushed with gas.

(4) During evaporation of the solvent, isothermal conditions are employed. Subsequently, ovens 1 and 2 are temperature programmed independently of each other, until a desired fraction of the sample is refocused and transferred from the uncoated precolumn to the column.

(5) By switching valves V_3 and V_6 (Fig. 2B) the uncoated precolumn is backflushed with gas. Meanwhile, the separation is proceeding in the analytical column. Before the liquid backflush procedure is initiated, the temperature in oven 1 is lowered below the boiling point of the backflush solvents.

(6) The uncoated precolumn is rinsed with the backflush solvents by switching valves V_4 and V_5 (Fig. 2C).

(7) Valves V_4 and V_5 are switched back to the initial position when the liquid backflush cycle is completed. The solvent residues in the uncoated precolumn are removed by increasing the temperature of oven 1.

(8) When the analysis is completed, valves V_3 and V_6 are switched back to the initial position. After lowering the temperatures of the ovens the system is ready for the next sample.

RESULTS AND DISCUSSION

System considerations

Two-dimensional GC is performed either with an in-line rotary valve between the columns or by pneumatic switching actuated from external valves (e.g., [17,18]). In-line rotary valves are easy to operate and the columns are mechanically separated from each other. However, frequently adsorption of sample solutes on non-glass surfaces of the valve is experienced and the large thermal mass of the valve causes thermal lagging compared with the oven temperature. These disadvantages are eliminated with pneumatic switching where the sample stream is directed between the columns by balancing the inlet pressures of the first and second columns via inert three-way connectors with a low thermal mass.

In this work we chose to construct a backflush system based on pneumatic switching. Apart from the reasons mentioned above, the main cause of abandoning our previous set-up [8], where an in-line rotary valve was utilized, was the occurrence of disturbances of the baseline after the solvent peak. When large volumes of liquid samples were injected, part of the solvent was trapped in the valve and slowly released as a large hump after the solvent peak. The solvent probably penetrated into the plastics of the rotor and/or the Vespel ferrule of the valve.

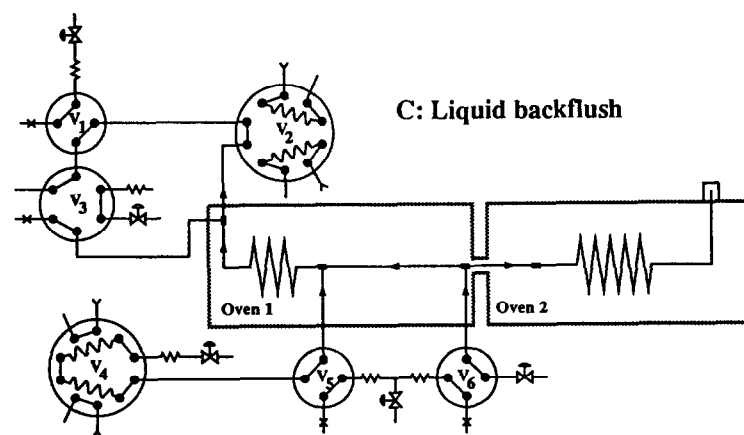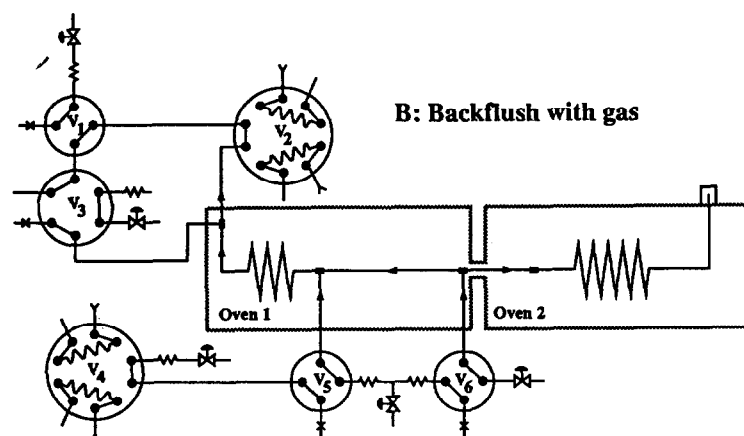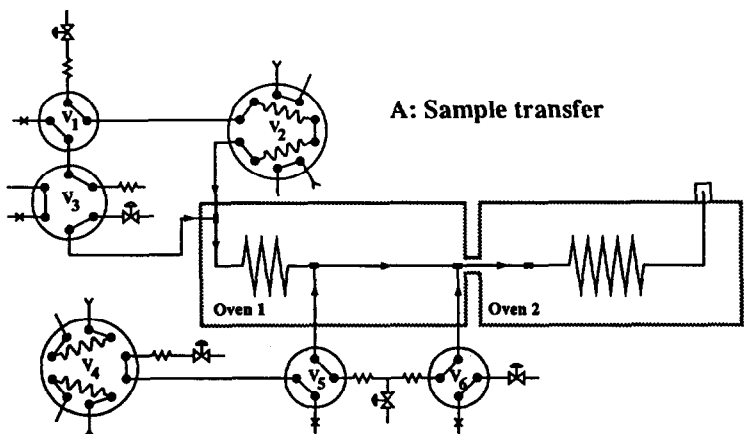In the present set-up, the vaporized sample is

Fig. 2. Operational steps of the liquid backflush system. (A) The sample is transferred to the uncoated precolumn; (B) the retention gap is backflushed with gas; (C) on-line liquid backflush of the precolumn while the analysis is proceeding in the analytical column.

only in contact with glass surfaces and the disorders mentioned above are absent. Balancing the pressures of a pneumatic system used for heart cutting is a delicate operation. Pressure balancing a Deans switch designed for backflushing with gas is, however, not critical [12]. When a desired fraction is transferred from the precolumn to the column (Fig. 2A), the inlet to the first column is simply opened to the atmosphere while the three-way connector between the columns is pressurized (Fig. 2B).

During the stage when the backflush solvents are transferred to the precolumn (Fig. 2C), the liquid causes a restriction of the precolumn and the gas velocity in the tubing between the two three-way connectors is slowed down. This velocity should not approach the diffusion velocity of the vapour of the solvent, otherwise solvent vapour will enter the analytical column. With the present set-up it was possible to introduce 500 μl of chloroform followed by 500 μl of hexane at a maximum speed of 7 μl/s ($P_3 = 4.1$ bar), using an inlet pressure of $P_5 = 1.3$ bar between the columns, without disturbing the flame ionization detector. When liquid samples are injected from valves, situated outside the oven, part of the transfer line between the valve and the column is not oven thermostated. It is preferable to keep this part as short as possible and also to backflush this transfer line with gas, in order to avoid solvent tailing caused by slowly released solvent residues from the cold part of the transfer line and the valve [19]. This solvent tailing is especially pronounced when solvents with a relatively high boiling point compared with ambient temperature are utilized.

A small part of the high-boiling solutes of the sample will remain in the cold part of the transfer line despite the backflushing with gas. This material can cause a memory effect during consecutive analyses, as it is likely that deposited solutes from the previous sample are released by the solvent of the next sample [20]. This has serious consequences when the samples have large concentration differences. With the liquid backflush system the cold part of the transfer line is rinsed with pure solvents, between each analysis, which removes the deposited solutes and the memory effects are thereby avoided.

In a repeatability test, R.S.D. values for absolute area counts of the order of 0.5% were obtained for twelve consecutive injections of even-numbered n-alkanes (C_{10}–C_{22}) diluted in hexane.

Direct injection of an olive oil sample

The high content of triglycerides in vegetable oils is known to contaminate GC inlets, causing problems in analysis of trace components. Methods for analyses of fats and oils by GC usually require laborious clean-up procedures such as saponification, extraction and sometimes also a preseparation by thin-layer or liquid chromatography [21]. A comparatively rapid and reproducible on-line coupled LC–GC method has been described and applied to the analysis of olive oils [21–23]. The triglycerides and other disturbing components were removed by LC prior to determination of sterols and wax esters by capillary GC.

Our prime interest with this sample was not to design a method for the determination of the quality of the olive oil, but to investigate how the liquid backflush system would perform with direct injections of a sample containing a large amount of high-boiling material. A 10-μl volume of a 1% solution of an olive oil followed by 10 μl of solvent wash were directly injected on to the precolumn with the same temperature programme (5°C/min) for the precolumn and the column. A very distorted chromatogram was obtained, as shown in Fig. 3A. The trace components of the sample interact with the high-boiling matrix during refocusing in the retention gap. This interaction leads to a slow transfer of the solutes from the precolumn to the column and extra-column band broadening occurs.

Changing the temperature program for the precolumn to 20°C/min while the temperature ramp of the column remained at 5°C/min dramatically improved the quality of the analysis (Fig. 3B). The high-boiling trace solutes are now transported at a much faster speed through the precolumn and band focusing is improved. Fig. 3C shows a chromatogram of the same sample after 100 consecutive analyses, using an on-line liquid backflush with 500 μl of chloroform and 500 μl of hexane during each analysis. As can be
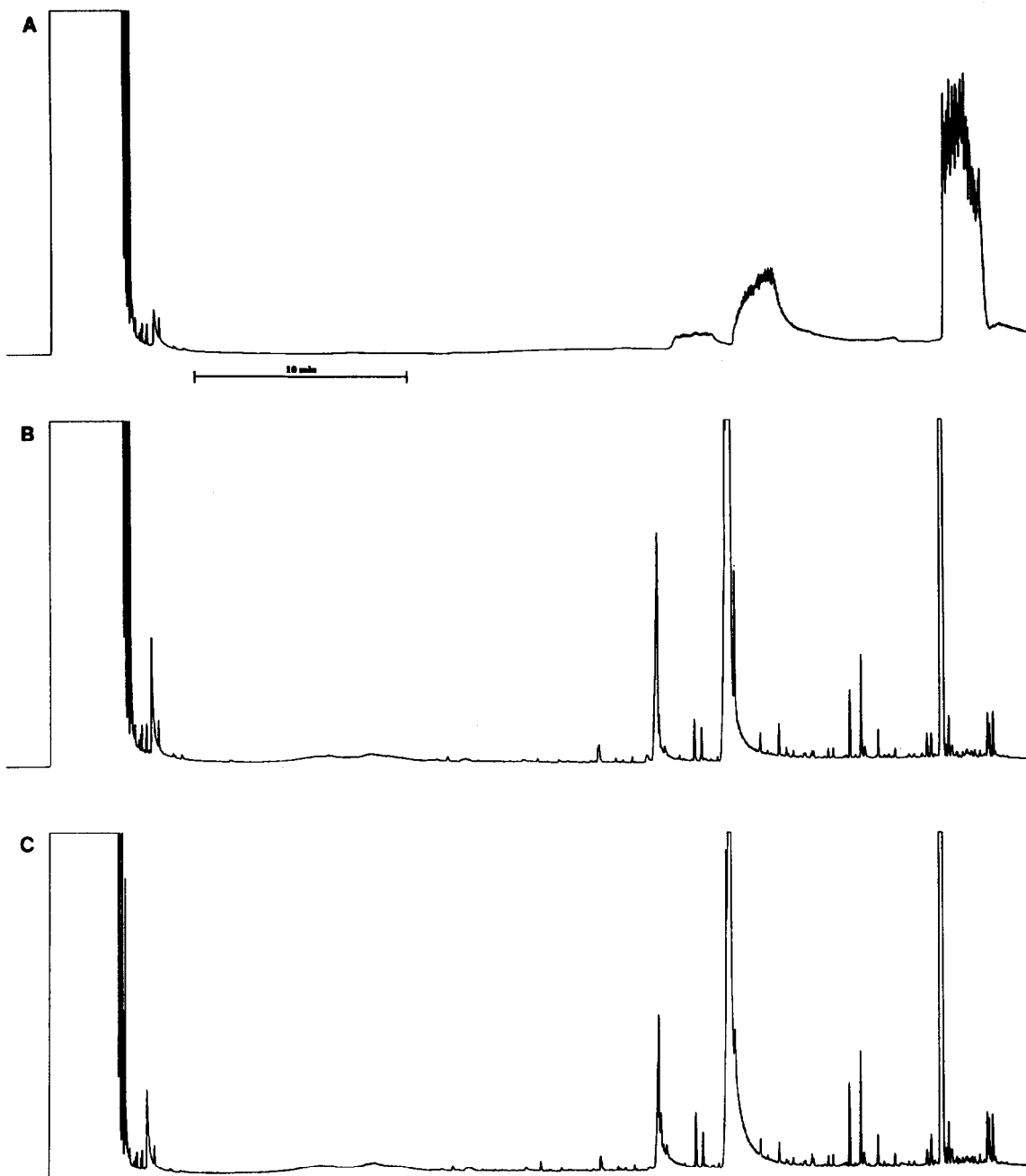
Fig. 3. Chromatograms of olive oil [1% (v/v) in hexane]. (A) Initial temperature 60°C. The same temperature programme (5°C/min) was employed for the uncoated precolumn and the column. (B) The retention gap was temperature programmed at 20°C/min (to 170°C) and the column at 5°C/min (to 300°C). (C) After 100 consecutive injections. Conditions as in (B) and a liquid backflush with 500 μl of chloroform and 500 μl of hexane during each analysis.

seen, the performance is still similar to the original one.

The activity level of the retention gap was

tested with injections of an n-alkane standard solution (with the same temperature ramp for the precolumn and the column) before, during

and after the experimental series. Depending on the amount of non-volatile material present in the retention gap, extra-column band broadening was observed. These experiments showed that most of the non-volatile material was removed by the solvent rinsing procedure. After 100
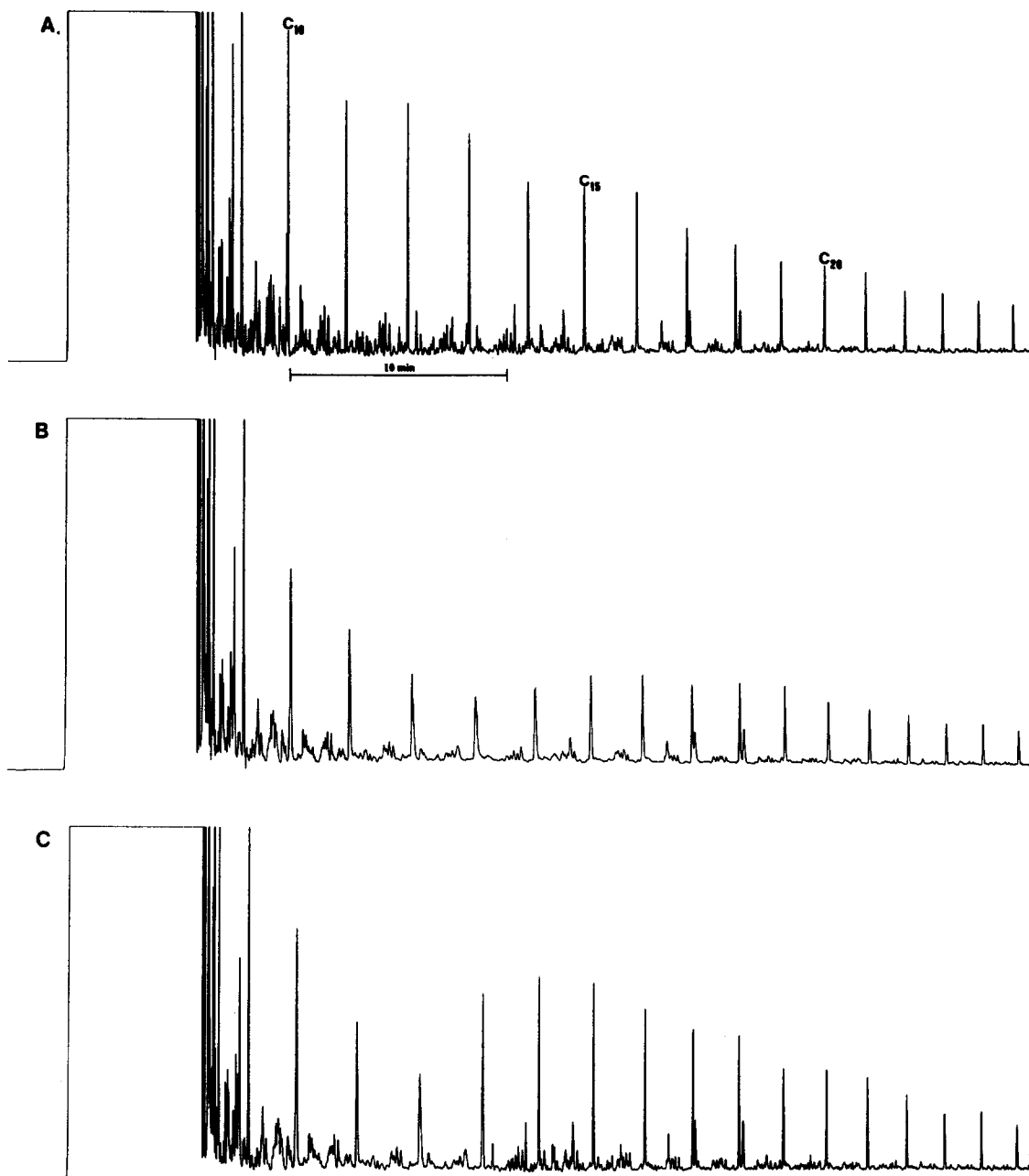

Fig. 4. Chromatograms of an Arabian light crude oil (100 μg/ml in chloroform). Influence of the temperature ramp of the precolumn on the system performance. (A) First injection; the uncoated precolumn and the analytical column were temperature programmed at 5°C/min (to 180 and 310°C, respectively). (B) After 100 injections; temperature programmes as in (A). (C) After 100 injections, where the uncoated precolumn was temperature programmed at 40°C/min and the column at 5°C/min.
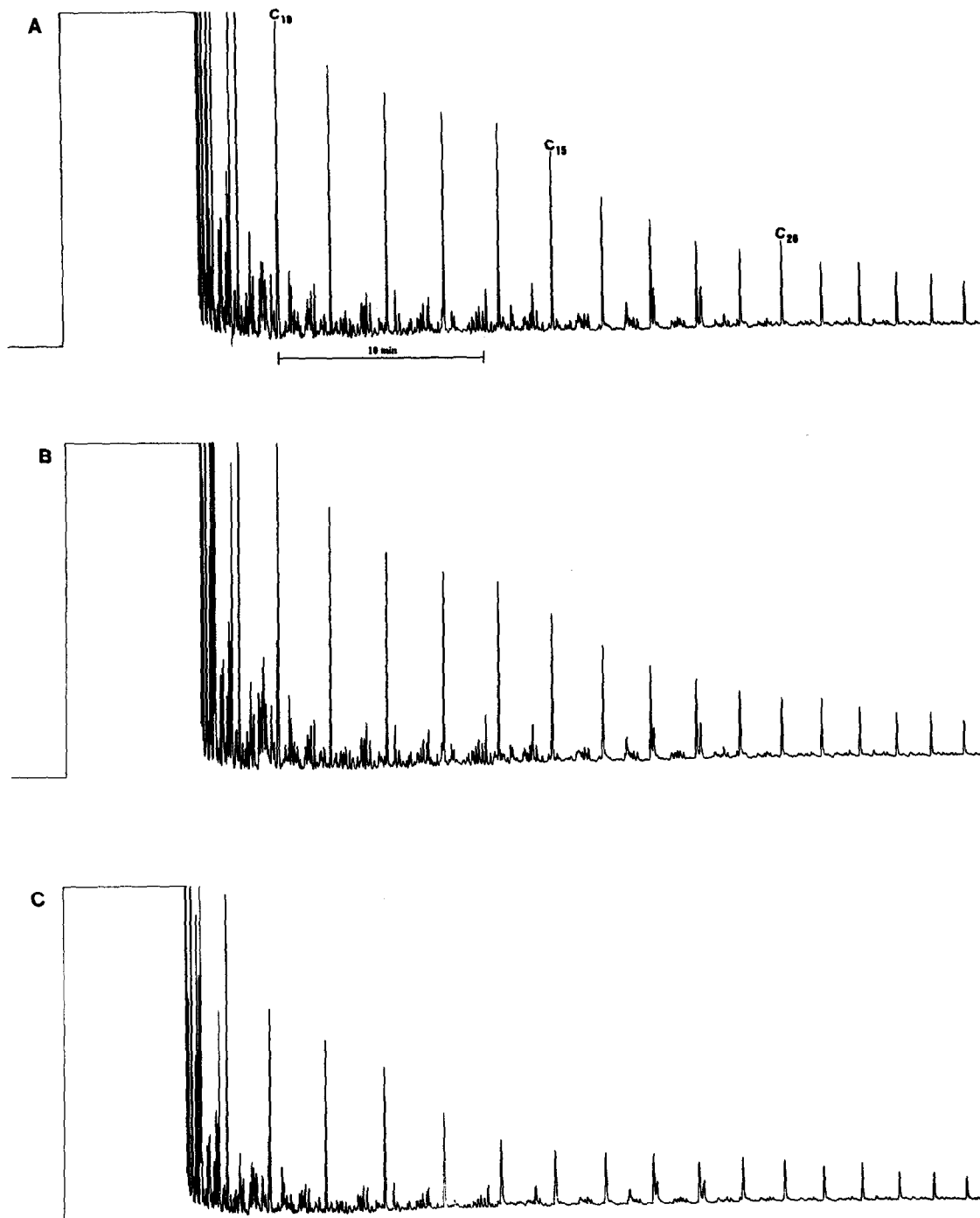
Fig. 5. Chromatograms of an Arabian light crude oil (100 µg/ml in chloroform). Influence of the liquid backflush on the long-term performance. (A) After 100 injections; (B) after 200 injections; (C) after 350 injections. Initial temperature, 60°C. The uncoated precolumn and the analytical column were temperature programmed at 5°C/min (to 180 and 310°C, respectively). A liquid backflush with 500 µl of hexane and 500 µl of chloroform was carried out during each analysis.

consecutive olive oil analyses with the liquid backflush procedure, the activity level of the retention gap was lower than after a single injection of the olive oil sample without a liquid backflush.

Another advantage of the double-oven system is that the final temperature of the uncoated precolumn and the coated column can be separately controlled. As the solutes of the sample are transported through the retention gap at a much lower temperature than the temperature when they start to migrate through the column, there is a risk that the column will become contaminated with non-elutable high-molecular-mass compounds if the final temperature of the precolumn is the same as that for the column. Contamination of the column with large amounts of triglycerides can be especially problematic in this respect as the volatility of these compounds is of a level where it can be difficult to elute them from the column. Transfer of these compounds to the column can be avoided by using a suitable lower final temperature of the precolumn than for the column.

Long-term analysis of an Arabian light crude oil

Capillary GC analysis of crude oils by on-column injection employing a short retention gap has been shown to suffer from a rapid decrease in separation power after a number of sample injections owing to the accumulation of non-volatile constituents [24]. In Fig. 4A, a chromatogram of an Arabian light crude oil, diluted 100 μg/ml in chloroform, is shown. When this sample was repeatedly injected, a gradual decrease in the chromatographic performance was experienced. Fig. 4B shows a chromatogram of the same sample after 100 consecutive injections. As can be seen, the peaks have become broad and much of the original resolution is lost. The analysis was performed without a liquid backflush using the same rate of temperature programming (5°C/min) for the uncoated precolumn and the analytical column.

In a following experiment, the precolumn was temperature programmed at 40°C/min while the temperature ramp of the column was kept at 5°C/min. The effect of the faster temperature ramp of the precolumn is shown in Fig. 4C. The resolution between the solutes that elutes after tridecane is restored, because the high-boiling solutes that are spread over the retention gap are transported to the column and cold trapped much faster when the precolumn is rapidly heated. Consequently, a higher retentive power of the precolumn caused by residual non-volatile material can be tolerated. The more volatile solutes, however, are reconcentrated by the solvent effect and by phase soaking [2], and these solute peaks remain broadened, despite the faster temperature programme of the precolumn. An attempt to restore the peak shapes also for these compounds was made by rinsing the precolumn with ethanol, chloroform and hexane. The efficiency was slightly improved but it was not possible to obtain the original performance. We believe that the prime cause of this is polymerization of the residual material.

The retention gap was exchanged and the same sample was again repeatedly injected. A liquid backflush with 500 μl of hexane and 500 μl of chloroform was now included during each analysis in order to remove the non-volatile material as fast as possible to minimize the risk of accumulation of polymerized residue. The useful lifetime of the retention gap was extended. The chromatographic efficiency was hardly affected during the first 100 injections, as can be seen in Fig. 5A, even though the same rate of temperature programming (5°C/min) was employed for the precolumn and the column. After 200 injections a slight decrease in the separation power became noticeable (Fig. 5B) and after 350 injections the decrease in efficiency was no longer acceptable (Fig. 5C). For the higher boiling solutes it was possible to restore the performance by using a faster temperature ramp of the precolumn, as described in the earlier experiment.

The retention gap was backflushed with a number of different solvents (acetone, carbon disulphide, chloroform, dichlormethane, diethyl ether, dimethyl sulphoxide, ethanol, hexane and isobutyl methyl ketone) in an attempt to dissolve the residue. The performance of the system was not restored, however. Finally, the retention gap
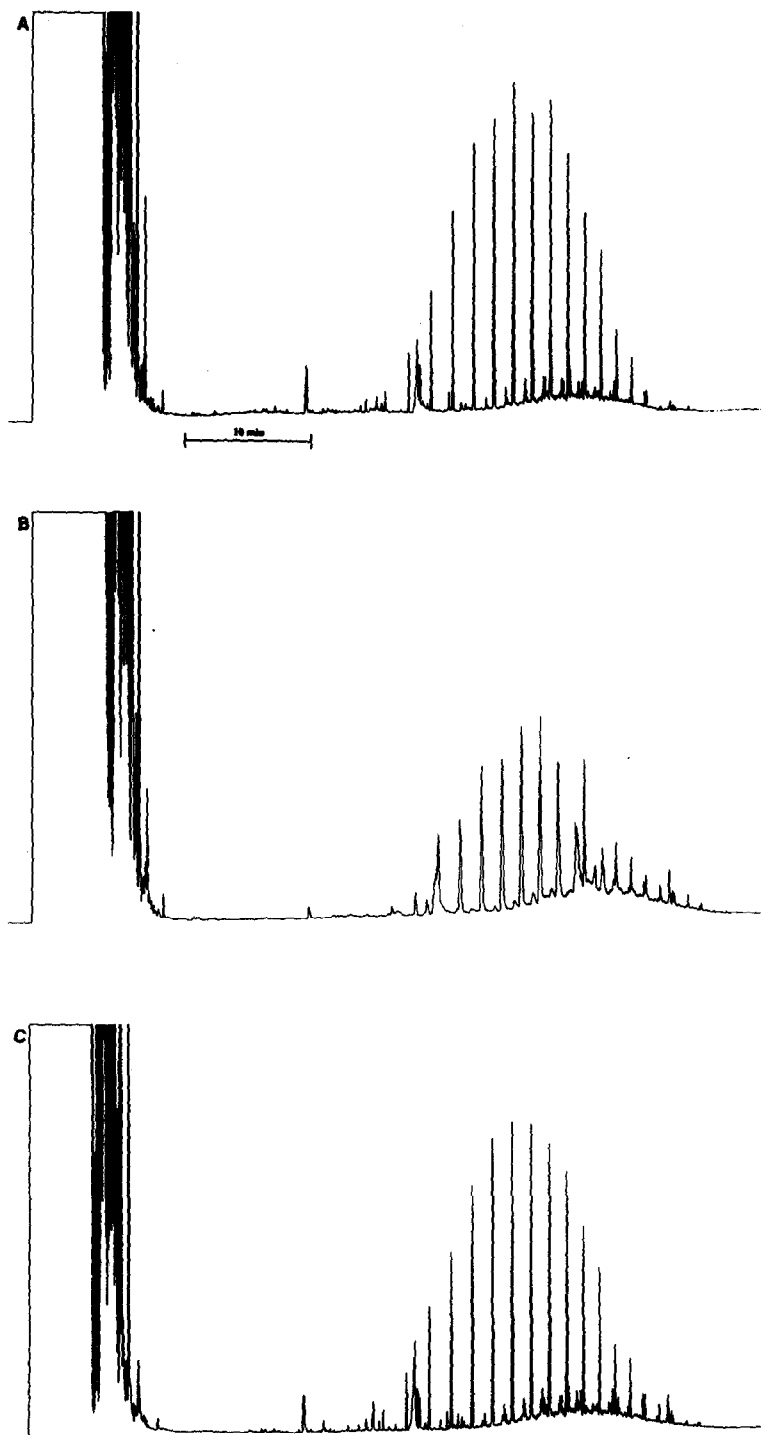
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Fig. 6. Chromatograms of shoe polish diluted in chloroform (500 μg/ml). (A) First injection. Retention gap and column temperature programmed at 5°C/min (to 170 and 300°C, respectively). (B) After 50 consecutive injections; conditions as in (A). (C) After 250 consecutive injections. Uncoated precolumn temperature programmed at 20°C/min and column at 5°C/min, liquid backflush with 500 μl of hexane and 500 μl of chloroform between every 25 injections.

was resilylated and rinsed on-line without any improvement.

Repetitive injections of a shoe polish solution

In another example, shoe polish diluted in chloroform (500 μg/ml) was filtered through a 0.5-μm Teflon filter and injected into the system. Fig. 6 shows the first chromatogram and a chromatogram after 50 consecutive injections without a liquid backflush and with the same rate of temperature programming for the precolumn and the column. As can be seen, a considerable decrease in separation power occurred. The performance was to a large extent restored after rinsing the precolumn with hexane and chloroform, but the original low activity level of the precolumn was not completely re-established. When the precolumn was temperature programmed at a faster rate than the column, chromatograms comparable to those from the first injection were obtained. As the solutes have a high boiling point and are reconcentrated by stationary phase focusing, some increase in activity level of the retention gap is tolerable, provided that an independently temperature programmed retention gap is used.

We continued to inject the sample with the faster temperature ramp (20°C/min) of the precolumn and executed a liquid backflush between every 25 injections. In Fig. 6C, a chromatogram after 250 consecutive injections is shown. The performance is still comparable to that in the first run.

CONCLUSIONS

Samples that contain non-volatile material contaminate uncoated precolumns, which rapidly affects the performance of the GC analysis. The useful lifetime of the retention gap is extended when a combination of a faster temperature programme for the uncoated precolumn than for the column and a liquid backflush of the precolumn are employed.

The main obstacle to dissolving the non-volatile material from the retention gap is polymerization of the residue. In this respect it is advantageous to remove the non-volatile constituents from the uncoated precolumn as fast as possible

after injection and reconcentration of the solutes of interest. Working conditions have to be established for each application where backflush solvents of different polarity have to be tested. The final temperature of the precolumn should be kept at the lowest level that allows reconcentration of the solutes, also in order to minimize the risk of polymerization of the residue.

Sample solutes that are reconcentrated by the solvent effect interact with the residues in the uncoated precolumn, causing broadened peaks. If, however, the sample solutes of interest have a relatively high boiling point compared with the solvent, a faster rate of temperature programming for the uncoated precolumn than for the column can be employed and these solutes are effectively refocused by cold trapping. When combined with a liquid backflush procedure that dissolves most of the non-volatile residue, a large number of samples can be analysed.

Column fouling caused by high-boiling solutes which, owing to the large difference in phase ratio, migrate through the uncoated precolumn but not through the coated column, can be avoided by keeping the final temperature of the precolumn oven below the final temperature of the second oven.

ACKNOWLEDGEMENTS

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