Universal Chromatography

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Chromatography is classified into gas chromatography (GC), supercritical fluid chromatography (SFC) and liquid chromatography (LC), depending on the physical properties of the mobile phase. Since the mobile phase in GC is a gas such as helium or nitrogen that does not have any interaction with analytes and carries them through the column, it is called a carrier gas. On the other hand, the mobile phase in LC dissolves analytes and will interact with them. Therefore, the mobile phase in LC significantly affects the selectivity of the separation. Although there are many options to improve the selectivity of the separation in LC, experience and knowledge are required for optimization. In SFC a high density gas is employed as the mobile phase, where the temperature and pressure in the column are higher than the critical values of the mobile phase employed. The mobile phase in SFC has intermediate properties between those in GC and in LC. **Table 1** compares physical properties of the mobile phase in three chromatographic modes. The density, viscosity and diffusion coefficients determine the solubility of analytes, permeability of the column and the speed of analysis, respectively.

The effect of temperature on retention behaviour in SFC on a thermodynamic basis has been discussed elsewhere, as well as thermodynamic retention behaviour in open tubular capillary SFC. It was found that LC-like or GC-like retention mechanisms were involved in the supercritical temperature region, depending on the column temperature and the inlet pressure.

Generally, separations in GC are performed using completely different systems from those in LC. The separation system in SFC is a hybrid between GC and LC. However, it should be noted that the temperature and pressure can control the physical properties of the mobile phase. If these two parameters are controlled, separations can be obtained in any three chromatographic modes using a single chromatographic system. Both plunger and syringe pumps can be used for this purpose.

Instrumentation

When an LC-like detector such as a UV detector is used, a two-pump system is favoured. The two pumps are operated in the pressure control mode, and the inlet and the outlet pressures are controlled independently, so that the pressure drop across the column can be controlled.

When a GC-like detector such as a flame ionization detector (FID) is used, the two-pump system is not applicable and a single-pump system is used. A low dead-volume restrictor is generally employed in this case to maintain an appropriate pressure and to avoid additional dispersion of analytes.

The oven should be capable of negative as well as positive temperature programming. Negative temperature programming is useful in SFC, as shown below.

Open tubular capillary columns with various immobilized stationary phases are commercially available. The smallest diameter commercially available is around 50 μm. On the other hand, packing materials with 3–50 μm particle diameters for LC can be used for packed capillary columns, and fused silica tubing or glass-lined stainless-steel tubing are available. Conventional packed columns (1–5 mm i.d.) are still used most often in SFC, but they do not fit into the compromise that constitutes universal chromatography.

Temperature Effect

Figure 1 shows the effect of column temperature on retention behaviour using a packed column and carbon dioxide or methanol as the mobile phase. The relationships between the logarithm of the retention factor of aromatic hydrocarbons, $\log k$, and the recip-

Table 1 Comparison of physical properties of the mobile phase in three chromatographic modes

Chromatography	*Density (g cm^{-3})*	*Viscosity (g cm^{-1} s^{-1})*	*Diffusion coefficient (cm^2 s^{-1})*
Gas chromatography	10^{-3}	$(0.5–3.5) \times 10^{-4}$	0.01–1.0
Supercritical fluid chromatography	0.2–0.9	$(0.2–1.0) \times 10^{-3}$	$(0.1–3.3) \times 10^{-4}$
Liquid chromatography	0.8–1.0	$(0.3–2.4) \times 10^{-2}$	$(0.5–2.0) \times 10^{-5}$

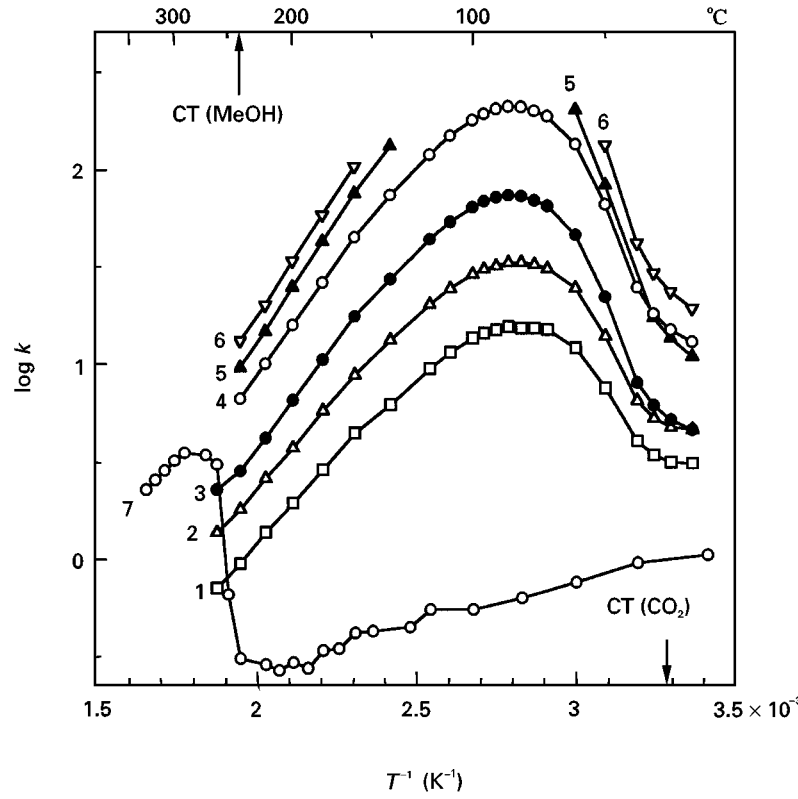

Figure 1 Relationships between the logarithm of the retention factor of aromatic hydrocarbons (log k) and the reciprocal of absolute column temperature (T^{-1}). Columns: Develosil ODS-5 (5 μm ODS), 150 × 0.5 mm i.d. (1–6) or 145 × 0.3 mm i.d. (7). Mobile phase: carbon dixoide (1–6) or methanol (7). Inlet pressure: 12 MPa (1–6) or 9.0 MPa (7). CT, Critical temperature. Samples: 1 = biphenyl; 2 = fluorene; 3 = *o*-terphenyl; 4 = pyrene; 5 = 9-phenylanthracene; 6 = triphenylene; 7 = pyrene. Detector: UV.

rocal of absolute column temperature, $1/T$, are shown in the figure with an inlet pressure of 12 MPa for carbon dioxide and 9.0 MPa for methanol. It should be noted that the pressure in the column is higher than each critical pressure, e.g. 7.38 MPa for carbon dioxide and 7.95 MPa for methanol. The critical temperatures of carbon dioxide and methanol are denoted as CT in the figure. At each higher critical temperature region, the retention factor increases with decreasing column temperature, while at each lower critical temperature region it decreases with decreasing column temperature. When the mobile phase is liquid, i.e. at a temperature lower than the CT, the retention factor increases with decreasing column temperature, as shown in the case of the methanol mobile phase.

It should be noted that the density of the mobile phase decreases with increasing temperature. The density of carbon dioxide is shown in **Figure 2**.

It is possible to distinguish a region in which the retention factor decreases with decreasing column temperature (SFC region) from one in which it increases with decreasing column temperature (high pressure GC region). The former region appears at lower supercritical temperatures, in which solvation of the analyte by the mobile phase is dominant, while the latter region appears at higher supercritical temperatures, in which the contribution of volatility is

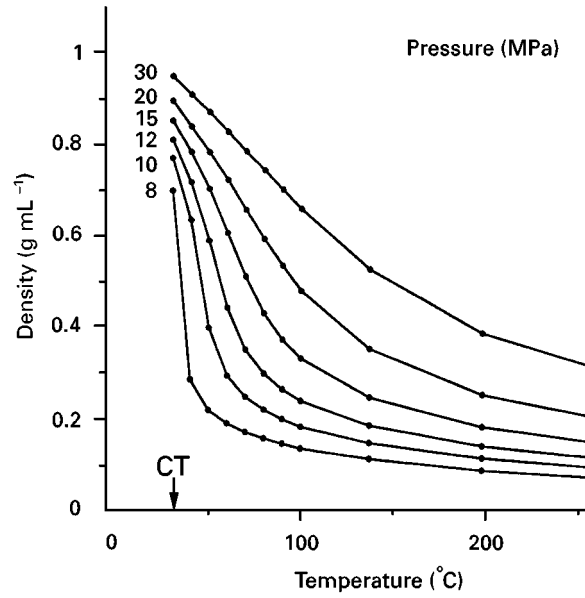

Figure 2 Density of carbon dioxide.

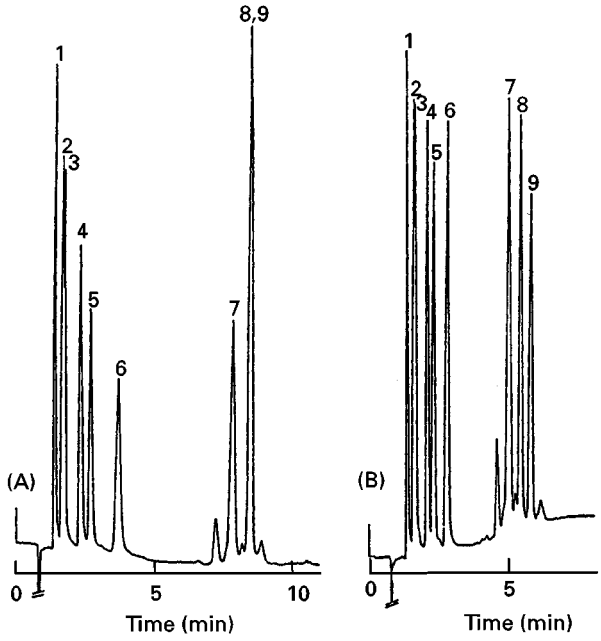

Figure 3 (A) Negative and (B) positive temperature programming of dialkyl phthalates on an SB-octyl-50 column. Column: SB-octyl-50 (5% *n*-octylmethylpolysiloxane), 10 m × 50 μm i.d. Mobile phase: carbon dioxide. Inlet pressure: 12 MPa. Samples: 1 = dimethyl; 2 = diethyl; 3 = diisobutyl; 4 = di-*n*-propyl; 5 = diisobutyl; 6 = di-*n*-butyl; 7 = diheptyl; 8 = di-2-ethylhexyl; 9 = dinonyl phthalate. Initial temperature: 130°C. Temperature programming: (A) $-10°C\ min^{-1}$ for 4 min, and $-5°C\ min^{-1}$ for the rest of the analysis; (B) $+10°C\ min^{-1}$ for 2 min, $+20°C\ min^{-1}$ for the next 4.5 min and kept at 240°C for the rest of the analysis. Wavelength of UV detection: 225 nm. (Reproduced with permission from Takeuchi *et al.* (1988). Temperature programming elution in capillary supercritical fluid chromatography. *Chromatographia* 25: 127.)

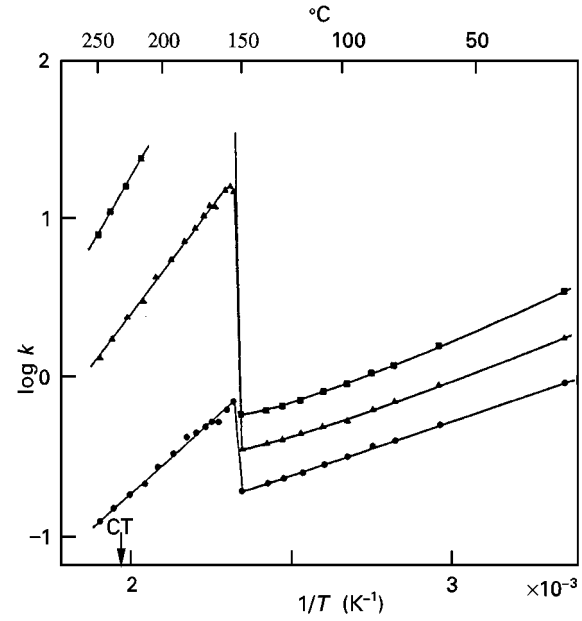

Figure 4 Log *k* versus $1/T$ for hexane mobile phase. Column: Develosil-60-10 (10 μm silica gel), 300 × 0.5 mm i.d. Mobile phase: hexane. Inlet pressure: 0.49 MPa. Outlet pressure: ambient pressure. CT, Critical temperature. Samples: ● = benzene; ▲ = naphthalene; ■ = anthracene. Wavelength of UV detection: 254 nm. (Reproduced with permission from Takeuchi *et al.* (1988). Micropacked column GC with vapor of organic substance as the mobile phase. *Chromatographia* 25: 994.)

dominant. In the intermediate temperature region, both contributions are involved. In addition, nonvolatile analytes cannot be eluted in the high pressure GC region, but they are eluted in the SFC and LC regions. It is clear that both SFC and high pressure GC separations can be demonstrated by changing the column temperature. Negative temperature programming is useful for the former, while positive temperature programming is useful for the latter mode.

Figure 3 demonstrates the separation of dialkyl phthalates using negative and positive temperature programming using an SB-octyl-50 open tubular capillary column and a UV detector. Both temperature programmes are started from the same temperature, i.e. 130°C. The programming rate is changed during the separation so that optimum resolution can be achieved in a reasonable time. The pressure is kept constant at 12 MPa during the separation. In Figure 3(A), SFC-like separation is demonstrated, while GC-like separation is demonstrated in Figure 3(B).

GC Using an LC System

In GC permanent gases are usually employed as the carrier gas. The vapour of an organic substance can also be used as the mobile phase for GC when the pressure in the column is lower than the vapour pressure at the operating temperature. In contrast, when the pressure in the column is higher than the vapour pressure, analytes are subjected to LC-mode separation. By controlling the column temperature and pressure, it is possible to demonstrate both GC and LC using a single LC system. It is convenient to use a micro-LC system because the heat capacity of micropacked columns of 0.2–0.5 mm i.d. is small.

Figure 4 shows the relationships between the logarithm of the retention factor and the reciprocal of the absolute column temperature when hexane and silica gel are used as the mobile and stationary phases. In Figure 4 the inlet pressure is 0.49 MPa, while the outlet pressure is atmospheric. The mobile phase is liquid when passing through the flow cell of the UV detector. The critical temperature and pressure of hexane are 234°C and 3.0 MPa, respectively. The critical temperature of hexane is denoted as CT in the figure. It should be noted that the pressure in the column is lower than the critical pressure. At higher

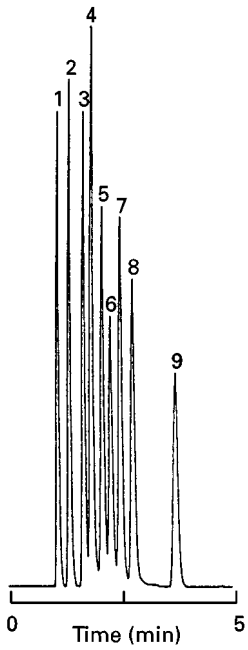

Figure 5 GC separation of alkylbenzenes at an inlet pressure of 0.49 MPa using hexane as a carrier gas. Column: Develosil-60-10 (10 μm silica gel), 300 × 0.5 mm i.d. Mobile phase: hexane. Inlet pressure: 0.49 MPa. Outlet pressure: ambient pressure. Column temperature: 205°C. Peaks: 1 = benzene; 2 = toluene; 3 = naphthalene; 4 = *o*-xylene; 5 = *n*-propylbenzene; 6 = mesitylene; 7 = sec.-butylbenzene; 8 = *n*-butylbenzene; 9 = *n*-amylbenzene. Wavelength of UV detection: 254 nm. (Reproduced with permission from Takeuchi *et al.* (1988). Micropacked column GC with vapor of organic substance as the mobile phase. *Chromatographia* 25: 995.)

temperatures linear relationships between log k and $1/T$ are observed, where the GC separation mode is involved. Since the applied pressure is lower than the critical pressure, the hexane vaporizes at a temperature lower than the critical temperature. When the outlet pressure is higher than the vapour pressure at the operated temperature, the hexane is liquid in the column and analytes are separated in the normal-phase LC mode; almost linear relationships between two parameters are observed. At some intermediate temperature the retention factor drastically changes with column temperature, where the state of the hexane in the column changes from liquid to gas, and both liquid and gaseous hexane exist at the boundary region in the column.

Figure 5 demonstrates GC separation of alkylbenzenes using hexane vapour as the mobile phase. The analytes are monitored by a UV detector at 254 nm. The mobile phase is liquefied while passing through a 60 cm × 70 μm i.d. capillary tube connecting the separation column and the flow cell of the detector. The capillary tubing is kept at ambient temperature.

GC Using LC Columns

Micropacked columns for LC can be applied to GC separation of hydrocarbons. When a glass-lined stainless-steel tube of 30 cm × 0.3 mm i.d., packed with 5 μm alkyl-modified silica, is employed as the separation column, a microvalve injector for LC should be used because of the large pressure drop across the column.

Figure 6 demonstrates the separation of C_6–C_{20} straight-chain hydrocarbons using carbon dioxide carrier gas, an octadecylsilica (ODS) column and an FID. The inlet pressure of carbon dioxide as the carrier gas is kept at 6.2 MPa. A volume of 0.02 μL of the sample dissolved in pentane is injected. The concentration is *c.* 2% (v/v) each, corresponding to *c.* 0.3 μg each of the injected amount. The column temperature is 35°C for the initial 5 min, programmed to 230°C at a rate of 5°C min^{-1}, and then held at 230°C. As far as alkyl-modified silica packings are employed, the SFC mode will be favoured for hydrocarbons with a carbon number larger than 15 because an increase in the background signal of the FID is observed at temperatures higher than 160°C.

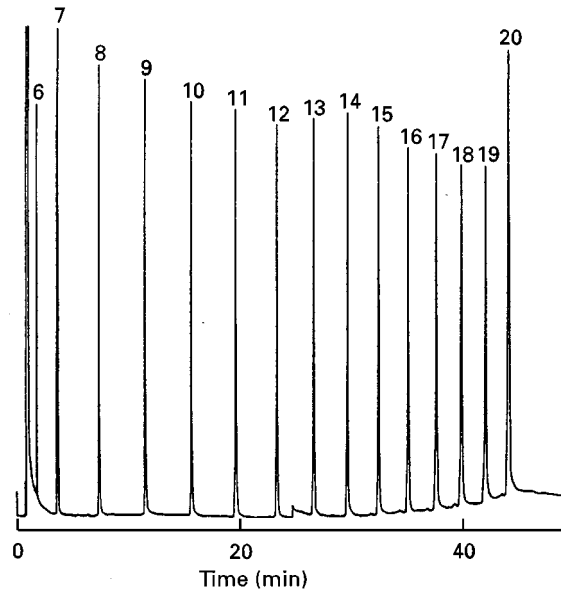

Figure 6 Separation of an artificial mixture of straight-chain hydrocarbons. Column: Capcell Pak C18 (5 μm ODS), 300 × 0.3 mm i.d. Mobile phase: carbon dioxide. Inlet pressure: 6.2 MPa. Column temperature: 35°C for the initial 5 min, then programmed at 5°C min^{-1}, held at 230°C. Peaks: straight-chain hydrocarbons (the numbers refer to the carbon numbers of the corresponding straight-chain hydrocarbons), *c.* 2% (v/v) each dissolved in pentane is injected. Detector: FID. (Reproduced with permission from Takeuchi *et al.* (1989). New approach to the GC separation of hydrocarbons by using LC-like microcolumns. *Chromatographia* 27: 183.)

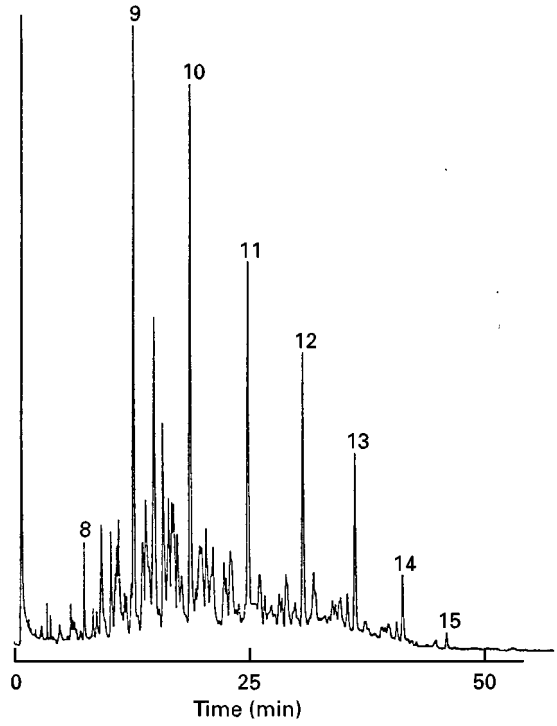

Figure 7 Separation of components of a kerosene. Operating conditions as in Figure 6, except for the sample and the temperature-programming rate. Column temperature: 35°C for the initial 5 min, then programmed at 3°C min^{-1}. Sample: kerosene diluted twice with pentane. (Reproduced with permission from Takeuchi *et al.* (1989). New approach to the GC separation of hydrocarbons by using LC-like microcolumns. *Chromatographia* 27: 184.)

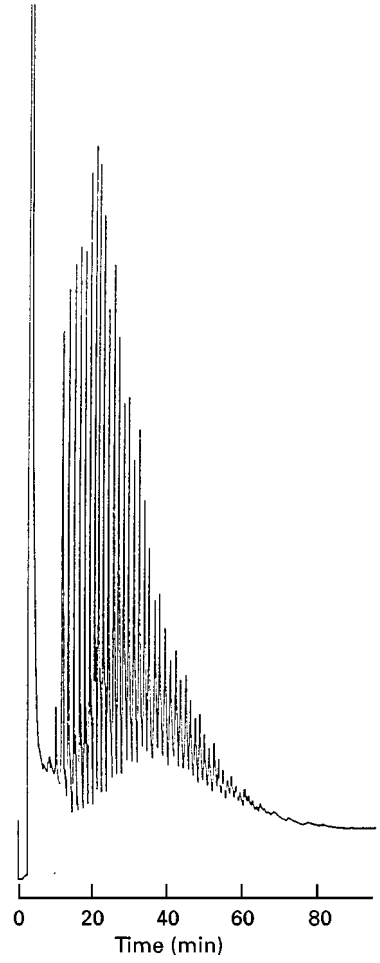

Figure 8 Pressure-programming SFC separation methylphenylsiloxane oligomers using diethyl ether as the mobile phase. Column: Develosil ODS-5 (5 μm ODS), 300 × 0.5 mm i.d. Mobile phase: diethyl ether. Initial inlet pressure: 4.4 MPa. Pressure programmed at 0.1 MPa min^{-1} for 10 min, 0.05 MPa min^{-1} for the next 10 min and 0.025 MPa min^{-1} for the rest of the analysis. Pressure drop: 0.49 MPa. Temperature: 225°C. Sample: OV-17 dissolved in tetrahydrofuran. Detector: UV (215 nm). (Reproduced with permission from Takeuchi *et al.* (1987). Retention behaviour in liquid and supercritical fluid chromatography using methanol or diethyl as mobile phase. *Chromatographia* 23: 932.)

Figure 7 demonstrates the separation of the components of a kerosene. The temperature-programming rate of the column is reduced to 3°C min^{-1}. The numbers in the figure correspond to the carbon numbers of straight-chain hydrocarbons. The results offer encouragement for the use of a micropacked column as a common separation column for GC, SFC and LC.

SFC Using LC Columns

Figure 8 demonstrates the pressure-programming separation of methylphenylsiloxane oligomers using diethyl ether as the supercritical fluid. The critical temperature, pressure and density are 194°C, 3.6 MPa and 0.264 g cm^{-3}, respectively. The initial inlet pressure is 4.4 MPa and the pressure is programmed at 0.1 MPa min^{-1} for 10 min, 0.05 MPa min^{-1} for the next 10 min and 0.025 MPa min^{-1} for the rest of the analysis. The pressure drop across the column is maintained at 0.49 MPa using a two-pump system. The temperature is kept constant at 225°C and the analytes are detected by UV at 215 nm.

Ether is toxic and flammable. Even a small leakage of the mobile phase should be avoided for safety reasons.

Multi-mode Separations in a Single Chromatographic Run

Changing the column temperature and the pressure by using a single system allows different modes of separation to be carried out. Capillary columns, packed or open tubular, facilitate the demonstration

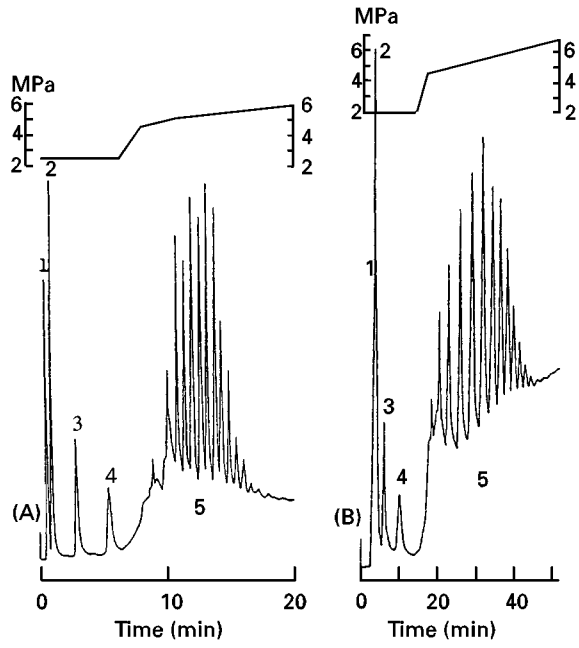

Figure 9 Multi-mode separation of aromatic hydrocarbons and styrene oligomers. Columns: (A) Develosil 100-5 (5 μm silica gel), 150 × 0.5 mm i.d.; (B) 13 m × 53 μm i.d., treated with 1 mol L^{-1} sodium hydroxide at 55°C for 2 days. Mobile phase: diethyl ether. Initial inlet pressure: programmed as shown in the figure. Pressure drop: 0.49 MPa. Temperature: 220°C. Samples: 1 = benzene; 2 = naphthalene; 3 = anthracene; 4 = pyrene; 5 = polystyrene A-1000. Wavelength of UV detection: 220 nm. (Reproduced with permission from Ishii *et al.* (1988). Unified capillary chromatography. *Journal of High Resolution Chromatography & Chromatographic Communications* 11: 801.)

of these different modes of separation with a single chromatographic system. This is because the capillary column can achieve excellent column efficiencies. While universal chromatography allows a number of combinations to be used, it does offer more restrictions, e.g. in terms of permitted column diameters and flow rates, compared with three separate instruments.

Figure 9(A) demonstrates the separation of a prepared mixture of aromatic hydrocarbons and styrene oligomers on a silica gel packed column using the vapour of diethyl ether as the mobile phase. The analytes are isothermally separated at supercritical temperature, and the pressure is programmed so that the aromatic hydrocarbons can be separated in the GC mode prior to the SFC separation of the styrene oligomers. The pressure drop across the separation column is kept at 0.49 MPa by using a two-pump system. The inlet and outlet pressures are controlled by a microcomputer. The hydrocarbons are separated at subcritical pressure; the inlet and outlet pressures are kept at 2.45 and 1.96 MPa, respectively. The styrene oligomers are then separated by pressure-programmed elution. The profile of the pressure programme is shown in the figure. This type of separation is of practical importance when the sample contains constituents with a wide range of volatility.

Figure 9(B) demonstrates the separation of the same mixture as in Figure 9(A) using an open tubular glass capillary column with 53 μm i.d. × 13 m. The active surface is produced by treating soda-lime glass capillary tubing with 1 mol L^{-1} sodium hydroxide aqueous solution at 55°C for 2 days. Nearly the same selectivity is observed between the open tubular and packed silica gel column.

Conclusion

Universal chromatography can perform the analysis of a variety of samples using a single chromatograph in which all separation modes can be selected. Universal chromatography also allows different modes of separation in series in a single run using a single chromatographic system. It allows the possibility of analysing a variety of samples with a wide range of volatilities in a single run by selecting multiple separation modes. For the more widespread use of universal chromatography appropriate detectors and stationary phases need to be developed.

See also: **I/Chromatography. II/Chromatography: Gas:** Theory of Gas Chromatography. **Chromatography: Liquid:** Theory of Liquid Chromatography. **Chromatography: Supercritical Fluid:** Theory of Supercritical Fluid Chromatography.

Further Reading

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