Review

Principles and applications of unified chromatography

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Abstract

The development and applications of unified chromatography (gas, supercritical fluid, and liquid chromatography in a single instrument) are reviewed. The principles, systems, and detectors of unified chromatography are discussed, describing the efforts which have been made in this approach to chromatography since the 1980s.

Contents

1. Introduction

Chromatographic separation modes [gas chromatography (GC), supercritical fluid chromatography (SFC) and high-performance liquid chromatography (HPLC)] are classified according to the physical state of the mobile phase (gas, supercritical fluid or liquid) in the column. However, there are no theoretical boundaries between them [1-7], and these distinctions are arbitrary, artificial, and counterproductive as Giddings pointed out as early as 1965 [1]. For

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example, fundamental chromatographic column theory, such as the Golay equation, can be applied to either GC, SFC or HPLC. The plate height of a column is a function of the velocity of the mobile phase which can be either gas, supercritical fluid or liquid.

In practice, the distinction between open tubular GC and SFC is blurred due to the use of the same type of columns and the same type of detectors. In packed-column SFC analyses, the use of binary or ternary mobile phases (CO_2 + liquid), sometimes near or even below the critical temperature of CO_2 has also blurred the distinction between SFC and HPLC, particularly when high-temperature HPLC is considered. Mobile phase strength programming (composition gradients) have become the most popular means of elution with the newer packed-column SFC instruments. Traditional GC and HPLC instruments have different types of columns, and different means of injection and detection in order to meet their own requirements, while SFC is more flexible in that it incorporates both GC and HPLC elements.

Fig. 1 shows the Van Deemter efficiency curves plotted for the various combinations of parameters under GC, SFC and HPLC conditions [6]. All pertinent dimensions and typical values of diffusion coefficients for the various mobile phases are considered. The Golay equation was used for open tubular columns, and the Knox equation was used for the packed columns; the commonly used regions of these curves are highlighted. Fig. 1 indicates that the optimum velocities for the different techniques are different, which is one of the major difficulties to be overcome in unified chromatography. Another major difficulty in unified chromatography is the lack of a simple detector which is compatible with all separation modes (GC, SFC and HPLC).

Towards the end of the 1980s, the concept arose of using a single chromatographic system to carry out separations in different modes [7–9].
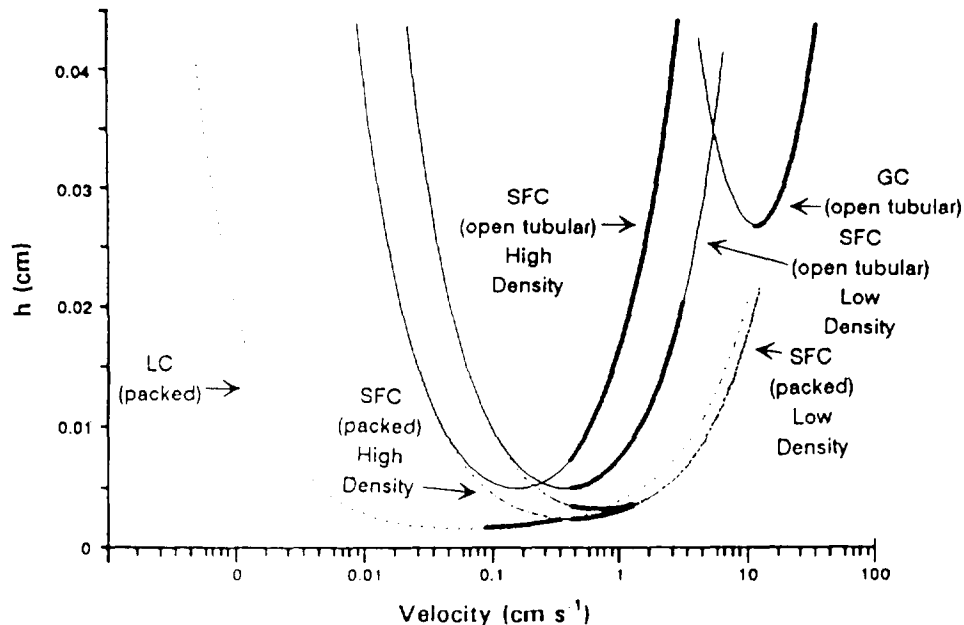

Fig. 1. Efficiency as a function of mobile phase velocity. Commonly used efficiency–velocity ranges are highlighted. Packed column conditions: 5-μm particles, values calculated from the Knox equation with $A = 1$, $B = 2$, $C = 0.05$ (for LC) and $C = 0.5$ (for SFC). Open tubular column conditions: 50 μm I.D. (SFC), 300 μm I.D. (GC), values calculated from the Golay equation with $k = 5$. Values for D_m were 10^{-5} cm^2 s^{-1} for liquid, 2·10^{-4} cm^2 s^{-1} for high-density supercritical fluid (100°C), 5·10^{-4} cm^2 s^{-1} for low-density supercritical fluid (100°C) and 10^{-1} cm^2 s^{-1} for gas. From Ref. [6]; © Chromatography Conferences.

Several research groups have described approaches to unified capillary chromatography in which LC, SFC and GC separations could be carried out by changing only the column temperature and the pressure using a single chromatographic system or by changing the mobile phase. The use of microcolumns in unified chromatography has distinct advantages. As Yang [7] suggested, the practice of GC, SFC and microcolumn HPLC becomes more similar as the column diameter becomes smaller; injector, column and detector may all be the same. Bartle et al. [10], in 1989, also pointed out that unified microcolumn chromatography allows high-resolution separations that make use of the same chromatographic components (injector, column and detector) for capillary GC, microcolumn SFC and HPLC [10]. Several research groups have demonstrated that the unified chromatography is achievable [8–24]. A variety of terms have been applied to the combination of different modes in a single system, such as *unified approach to chromatography* [7], *unified capillary chromatography* [8], *unified microcolumn chromatography* [10], *unified fluid chromatography* [11], *unified chromatography* [22], *and unified GC and SFC* [23], *unified chromatograph for GC, SFC, and LC* [25].

Unified chromatography can not only lead to new techniques for the miniaturization of chromatography, but also produce new separation methods for many samples which are impossible or difficult to analyse in a single run with a single mobile phase.

2. Separation systems

2.1. Exploration of sequential analysis

In 1987, Pentoney et al. [9] made the first approach to sequential analysis with one injection and a single column, but using two analytical systems. They demonstrated a method of analyzing mixtures containing both volatile and non-volatile compounds using one injection and one column by transferring the 19.5 m × 100 μm I.D. fused-silica open tubular column from a GC instrument to an SFC instrument. Injection of the sample was accomplished by quickly dipping the end of the capillary column into the sample solution, and reconnecting the column to the gas chromatograph. The volatile portion of the sample was separated by GC using temperature programming up to 250°C following rapid cooling of the oven. The column was then removed and placed in a SFC instrument; the non-volatile portion was separated by SFC with pressure programming.

2.2. Single-substance mobile phase systems

Pioneering work on unified chromatography was carried out by Ishii et al. [12]. In 1987, they constructed a system using two pumps and a packed column placed in an oven (shown in Fig. 2). They investigated the effects of column temperature and pressure on the retention behaviour of aromatic hydrocarbons with methanol or diethyl ether as the mobile phase; by changing pressure and temperature the system could be operated under LC and SFC conditions. Both positive and negative temperature programming and pressure programming were applied to separate styrene oligomers, and pressure programming in the separation of styrene oligomers, non-ionic detergent and methylphenylsiloxane [12]. Investigations were carried out of the effects of column temperature on the retention behaviour of aromatic hydrocarbons and dialkyl phthalates under SFC conditions, and it was pointed out that negative temperature programming can partly replace pressure programming and that positive temperature programming is applicable to solutes with appropriate volatility [13].

Later, the Ishii group employed packed capillary columns (0.3–0.7 mm I.D. glass-lined stainless-steel tubing packed with micro particles) and fused-silica open tubular columns (50 μm I.D.) and demonstrated sequential GC–SFC analysis in a single instrument. These separations were carried out in series in a single chromatographic run by controlling the temperature and pressure of methanol or diethyl ether mobile phase [11]. Separate pumps were used to control the inlet
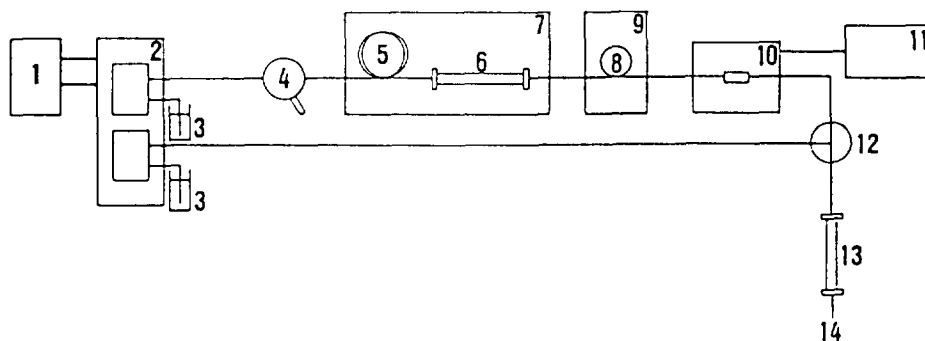
Fig. 2. Diagram of a unified chromatography system with a single-substance mobile phase (diethyl ether). 1 = Microcomputer; 2 = CCPM HPLC pump; 3 = solvents; 4 = injection valve; 5 = preheating capillary; 6 = analytical column; 7 = GC oven; 8 = cooling capillary; 9 = water bath; 10 = UV detector; 11 = chart recorder; 12 = Tee union; 13 = restriction column; 14 = vent. From Ref. [12]; © Chromatographia.

and outlet pressures, so that the pressure drop across the separation column could be kept constant. Separations were thus performed in series in a single chromatographic run, and so that mixtures containing components with wide ranging volatilities could be analysed [8].

Ishii and Takeuchi [8] also extended the concepts of unified chromatography by showing that the separation mode can be selected by changing the oven temperature and the pressure in the column; mobile phase delivery, injection, separation and detection systems must then be developed so that the different modes (LC, SFC and GC) can be selected by using a single chromatographic system; moreover, the different mode separations can be carried out in series in a single chromatographic run by the careful selection of the operating conditions.

Columns packed with microparticles can not only be used in HPLC and SFC, but can also be used in GC for the high-resolution separation of low-molecular-mass compounds [15–19]. Takeuchi and co-workers used a glass-lined stainless-steel tube of 30 cm × 0.3 mm I.D., packed with 5-μm alkyl-modified silica as GC separation column with carbon dioxide or nitrogen as the carrier gas [18]. They also used the vapour of organic substances (hexane or diethyl ether) as carrier gas with a 30 cm × 0.5 mm I.D. glass-lined stainless-steel tubing packed with Develosil 60-10 silica gel followed by UV detection [19].

Gemmel et al. [20,21] investigated consecutive gradients in chromatography using pentane and dioxane as mobile phases. They started separations under sub-critical (gaseous) conditions and increased the pressure until supercritical conditions were obtained; volatile components of a given sample could be separated under high resolution and the remainder of the sample can be separated by programming the temperature to values much lower than those in high-temperature GC. Composition gradients may also be combined with simultaneous temperature gradients.

Steenackers and Sandra [22] demonstrated unified chromatographic separations using 50 and 100 μm I.D. packed capillary columns packed with 5- or 10-μm particles to achieve highly efficient separations. They used a 50 cm × 50 μm I.D. column packed with 5-μm Spherisorb ODS-2 to separate C_8 and C_{20} hydrocarbons and pointed out that because of the high pressure drop and the low density of carbon dioxide the conditions under the initial pressure should be considered as high-pressure GC rather than SFC.

The GC conditions in the single-substance mobile phase systems were not normal due to use of a restrictor post-column (high-pressure GC with CO_2 as mobile phase). However, lower optimum velocities due to lower diffusion of CO_2 (higher viscosity and higher density than that of normal gases) gave longer retention and wider peaks.

2.3. Multi-substance mobile phase systems

Yang's group carried out the pioneer investigations using different mobile phases in a single separation run [23,24]. Davies and Yang [23] constructed a unified high-pressure GC and SFC instrument (shown in Fig. 3). They employed a rotary valve to select mobile phases with a short frit restrictor following the column. They introduced high-pressure helium mobile phase from a cylinder for GC to match the pressure drop across the combined column and frit restrictor. They also investigated the use of carbon dioxide at low pressure as GC mobile phase.

Davies and Yang [23] described how sequential GC and SFC could be performed with helium mobile phase at high pressure for GC and carbon dioxide mobile phase for SFC using a 10 m × 100 μm I.D. open tubular column. The inlet of the open tubular column was directly connected to the injection valve through a heated block beneath the injection valve. The theoretical aspects of this technique were also investigated with respect to mobile phase characteristics, optimum linear velocities, and peak capacities. The GC section of the single separation run was carried out with helium at high pressures (158 atm; 1 atm = 101 325 Pa) with temperature programming and the SFC section was performed with supercritical fluid carbon dioxide under pressure programming, and a constant temperature (100°C).

Liu and Yang [24] demonstrated sequential high-pressure GC and SFC analyses with microbore packed columns. One column (15 cm × 0.75 mm I.D.) was packed with 5-μm macroporous polymer by slurry packing, the second (10 cm × 1 mm I.D.) packed with 5-μm Delta bond octylsilica particles. In order to achieve desirable mobile phase linear velocities in both GC and SFC, they investigated Van Deemter plots for high-pressure GC and SFC with the polymer column and pointed out the importance of choosing a restrictor with the proper flow restriction to produce desirable linear velocities under both GC and SFC conditions. They also discussed sample injection with high-pressure helium in GC mode.
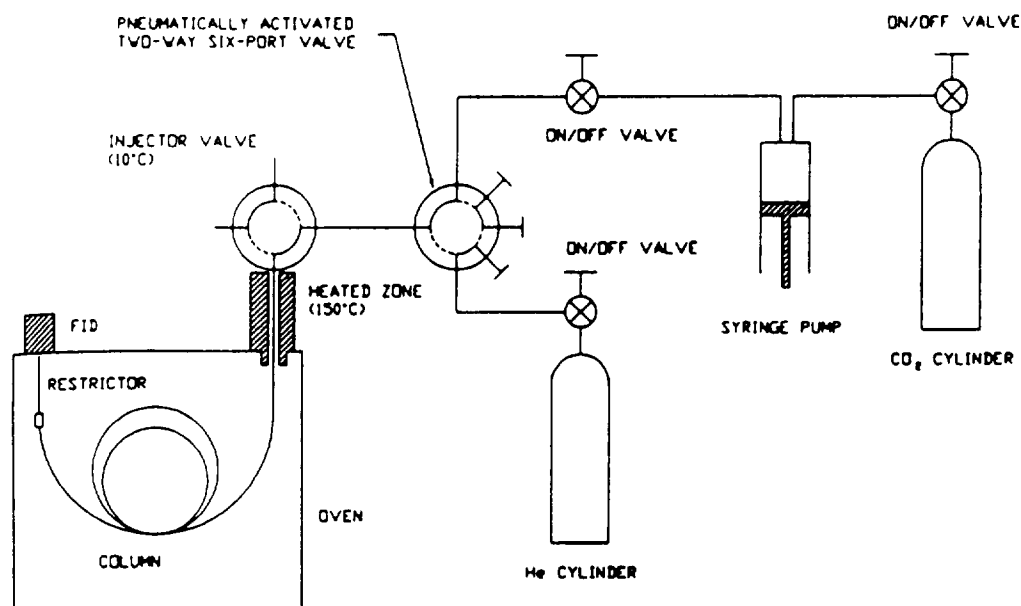

Fig. 3. Diagram of a unified GC and SFC system with two substances as mobile phases (high-pressure helium and carbon dioxide). From Ref. [23]: © Anal. Chem.

2.4. Independent optimization systems

In order to achieve the convenience of optimization of the linear velocities in both GC and SFC runs, Robinson et al. [26] constructed a unified chromatographic system with a rotary valve post-column (shown in Fig. 4) to direct the flow of column effluent to the flame ionization detector through a length of 50 μm I.D. fused-silica tubing, R1, during the GC separation or through a frit restrictor, R2, during the SFC separation. This arrangement allowed the system operating parameters of temperature and pressure programs and linear velocities to be optimized independently of each separation mode. They used this system with a 10 m × 100 μm I.D. open tubular column for GC, SFC and sequential GC–SFC analyses. The GC section of the run was carried out with helium at normal carrier gas pressures and followed by the SFC separation with carbon dioxide mobile phase at optimized linear velocities.

Tong et al. [27] later used 50 μm I.D. open

tubular columns for GC, SFC and sequential GC–SFC analyses to achieve high-resolution analysis. They improved the injection technique by limitation of the dead volume at the connection between the injector and the inlet of the retention gap and the connection between the outlet of the retention gap and the column. They also investigated the potential loss in separation efficiency resulting from the inclusion of the valve post-column during SFC and found that only a small change in separation efficiency resulted from inclusion of a valve with a 0.19-μl rotor slot volume. Separation efficiencies in sequential GC and SFC analyses by using 100 and 50 μm I.D. open tubular columns were compared.

Injection in GC mode has advantage that the solvent can be pre-separated from solutes in the retention gap or the inlet of a packed capillary column. The volume of the slot in the rotor of the injection valve (minimum 60 nl) imposes limitations so that the initial plug of the sample injected without splitting under SFC or HPLC
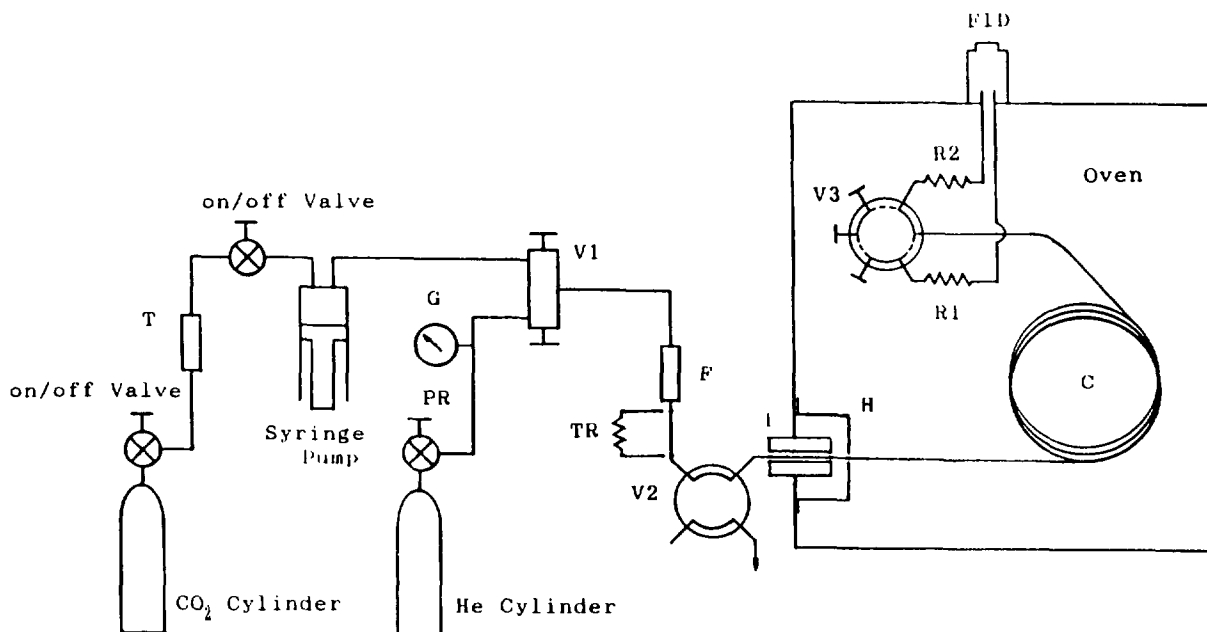

Fig. 4. Schematic diagram of a unified GC–SFC system. C = Analytical column; F = frit filter union; G = pressure gauge; H = heat-resistant casing; I = injection heater; PR = high-pressure regulator; R1 = linear restrictor; R2 = frit restrictor; T = activated charcoal trap; TR = transforming restrictor; V1 = two-stage three-way valve; V2 = injection valve; V3 = rotary valve. From Ref. [28]; © J. Microcol. Sep.

conditions may be too long, resulting in band broadening. In sequential GC–SFC or GC–HPLC analyses, the solutes pre-separated under GC conditions can be focused by the supercritical fluid or liquid mobile phase due to their higher solubilities, so that a larger injection volume can be introduced without loss of resolution.

Later, Tong and Bartle [28] recognised that unified chromatography using different substances as mobile phases, like all other coupled techniques, requires a suitable interface due to the different properties of the mobile phases. Pressure, density and optimum flow-rates during GC, SFC and HPLC may be very different from each other. Because of this, there is an intermediate stage during the mobile phase change from one to another. Peaks from components remaining in the column may be broadened during the mobile phase transformation. They investigated the interface problems by both theoretical study and experimental work. They described a shorter mode-transformation process and used the Golay equation to demonstrate the variation of plate height with mobile phase velocity at column mid-length point during the transformation from helium GC to CO_2 SFC (Fig. 5). They pointed out that such band broadening is caused by the violent variation of the linear velocity in the column during the mobile phase change period. Fig. 5 shows that the maximum over-velocity in the 50 μm I.D. column, u_{2max}, is smaller than that in the 100 μm I.D. column, u_{1max}, due to the greater restriction of the smaller-I.D. column. For further restriction of the over-velocity, a transforming restrictor (30 cm × 24 μm I.D. fused-silica capillary) was inserted between the frit filter union and the injection valve (Fig. 4). The system was further improved by reducing the replaceable volume (using smaller-diameter stainless-steel tubings and smaller inner volume valve) and by using low oven temperature and low initial CO_2 pressure during the mobile phase transformation period. Band broadening is greatly restricted through the improvement and the optimization of operation conditions [28].
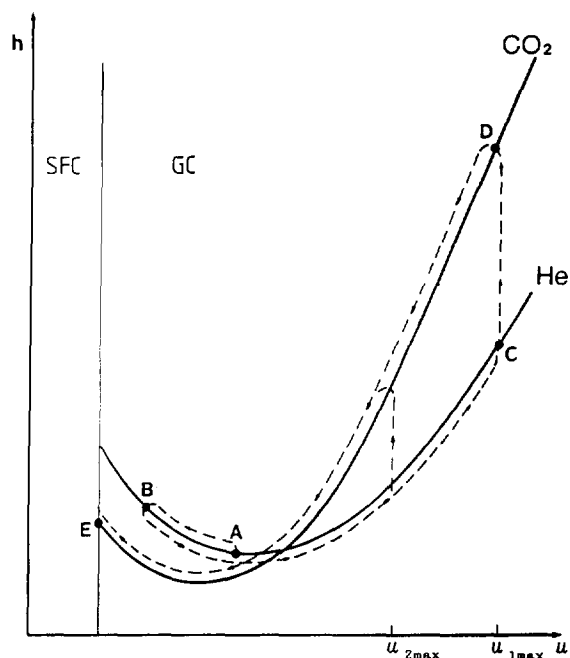
Tong et al. [29] constructed a unified

Fig. 5. Graph of variation of plate height (h) with mobile phase velocity (u) at mid-length point during mobile phase transformation. A = Point corresponding to normal GC conditions; B = point corresponding to the lowest helium velocity, after the gas supply is closed, but before carbon dioxide is introduced; C = point at which the velocity for the helium mobile phase increases to a maximum due to compression by carbon dioxide; D = point at which the mobile phase becomes gaseous carbon dioxide; E = point where the mobile phase approaches supercritical carbon dioxide. From Ref. [28]; © J. Microcol. Sep.

chromatograph with different arrangements for the separations of mixtures relevant to petrochemical analysis. GC, SFC, HPLC or sequential GC–SFC on open tubular or packed capillary columns could be carried out. Detection was either by flame ionization or UV absorption detection. A specially machined rotary valve which allows a maximum operating temperature up to 250°C was installed in the oven to appropriately direct eluate from the column. Analyses of motor gasoline by GC, saturates of an Arabian heavy atmospheric residue by SFC with open tubular columns, and coal tar by both SFC and HPLC with a packed capillary column were carried out by using this chromatograph.

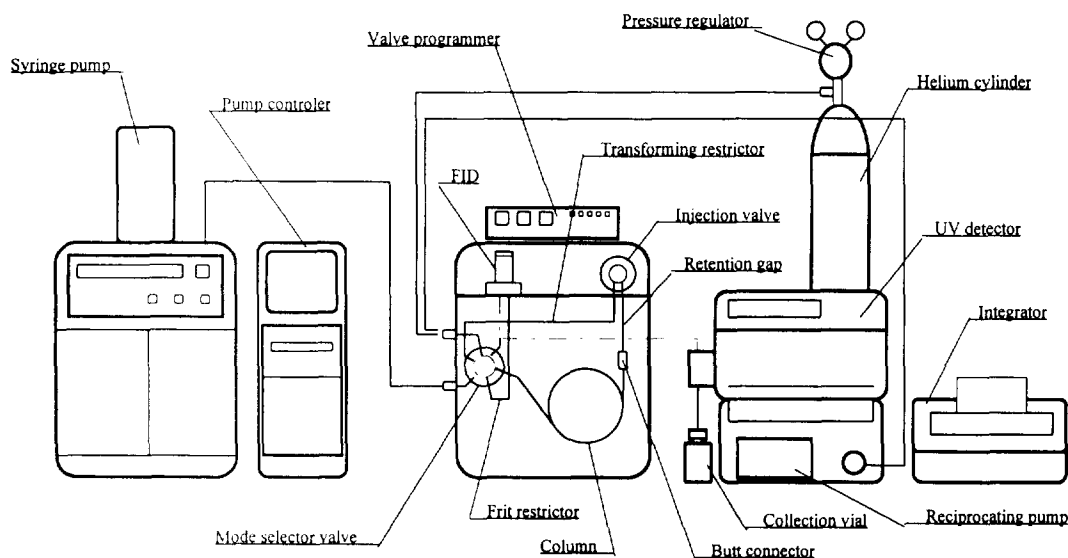Recently, Tong et al. [30] constructed a state-

Fig. 6. Schematic diagram of the state-of-the-art unified GC, SFC and HPLC chromatograph.

of-the-art unified chromatograph with a novel switching arrangement (shown in Fig. 6). This chromatograph employed only one six-port rotary valve to select mobile phase and direct column effluent to the detector. Change from one chromatographic mode to another was considerably simplified. Band broadening during mobile phase transformation was greatly restricted because the replaceable volume for mobile phase transformation was reduced due to the reduction of the number of valves and connecting tubings. Except for the injection valve, all elements which affect the transforming processes are installed in the oven to accelerate the various processes during the change from one mode to another, especially after HPLC analysis.

When helium supply tubing is connected to the system and a 50 μm I.D. fused-silica tubing (dashed line) is used between the mode selector valve and flame ionization detection (FID), either GC or SFC can be carried out by appropriately positioning the mode selector valve. Valve injection onto an open tubular column (even 50 μm I.D.) in GC mode is appropriate and high resolution can be achieved. This procedure avoids the problems of quantitative reproducibility for wide volatility range samples inherent in the split injection technique, and the

operating inconveniences with normal splitless injection.

If liquid supply tubing is connected to the system and the inlet of the UV detector is connected to the mode selector valve (dashed line), HPLC or SFC can be performed by appropriately positioning the mode selector valve. High-resolution analyses by SFC on both open tubular and packed capillary columns with FID detection and by HPLC on packed capillary columns with UV detection were achieved. The arrangement allows the flow eluent from the column to be directed either to the FID through the frit restrictor in SFC mode or to the UV–visible detector through fused-silica tubing to waste in HPLC mode. A high-efficiency packed capillary column prepared in-house [30,31] was installed in the oven. Two 30 cm × 50 μm I.D. fused-silica capillaries are connected to the two ends of the column by means of two Valco unions as inlet and outlet of the column without retention gap and butt connector. The column was packed with either 5-μm Spherisorb ODS-1, ODS-2, C_{18} (Phase Separations, Queensferry, UK) or 10-μm LiChrosorb Diol (Merck, UK); the column tubing material was 250 μm I.D. fused silica (Composite Metal Services, UK). The cell of the UV–visible detector has a volume

of 0.38 μl and is fabricated from a piece of 200 μm I.D. fused-silica tubing. The polyimide coating was removed over a 10 mm length, which is positioned in the slot of the UV–visible detector. A fused-silica capillary with 50 μm I.D. × 196 μm O.D. is used as a connection tubing between the mode selector valve and the inlet of the UV–visible detector. The end of this connection tubing is inserted into the inlet of the cell up to the cell window to reduce the dead volume at the connection. The outlet of the cell is positioned to waste. The frit restrictor is connected to the FID system in the same way as described above.

If the column outlet is directly connected to the inlet of the UV detector and the outlet of the UV detector is connected to the mode selector valve, the arrangement for HPLC with UV detection, or SFC with UV followed by FID is achieved. The outlet (50 cm) of the packed capillary column is directly inserted into the cell of the UV detector and the outlet of the UV detector is connected to the mode selector valve, so that the eluent flow from the UV detector can be either directed to FID during SFC analysis or through fused-silica tubing to waste for HPLC. For SFC using an organic supercritical fluid or modified carbon dioxide as mobile phase, FID is used as a heater to protect the restrictor from blockage.

3. Detection methods in unified chromatography

So far, there is no detector which is suited to all kinds of chromatographic separation modes. Mass spectrometry (MS), Fourier transform infrared (FT-IR) spectrometry, (UV) absorption detection, flame ionization detection (FID), thermionic detection (TID), flame photometric detection (FPD) and ion mobility spectrometry (IMS) have been investigated as detection means coupled with either GC, SFC or HPLC. Commonly, each detection can be very successfully applied to one or two separation modes.

3.1. Flame-based and miscellaneous detection

FID is widely used in GC and SFC. In the 1980s, efforts were made to interface FID with

HPLC including by using a belt interface and by direct connection. McGuffin and Novotny [32] designed and constructed a FID system for micro-LC using two flames to detect large molecules. Due to the very low flow-rates of micro-LC, it is possible to connect the column to the FID system directly. The use of a microcolumn can be an alternative means to reduce interference and instability in FID, while maintaining the universal detection capability. The total effluent can be introduced into the flame without an interface. However the organic solvents commonly used in HPLC create a large background ion current, upon which it is difficult to detect solutes. Some inorganic solvents, such as carbon disulphide and water, do not create a significant negative ion current in the flame and other small organic molecules that are not sensitively detected may serve as suitable mobile phase modifiers with limited concentration ranges [32,33].

McGuffin and Novotny [32] also constructed and investigated a TID system [32] (Fig. 7) and
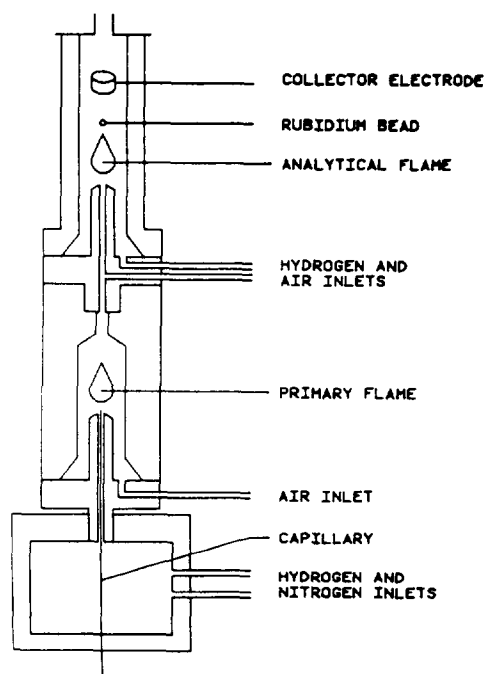

Fig. 7. Diagram of the thermionic detector for microcolumn liquid chromatography. From Ref. [32]; © Anal. Chem.

an FPD system for microcolumn analyses. The dual-flame construction of the thermionic detector minimizes the background ion current created during combustion of the column eluent. This detector is compatible with a wide range of solvents which contain only carbon, hydrogen and oxygen. In contrast, water, acetonitrile and methylene chloride caused a substantial increase in the background ion current. These solvents could be tolerated in concentrations up to 15–20% in the mobile phase.

3.2. Ion mobility detection

Hill [34] has suggested the use of ion mobility spectrometry as a detection method for unified chromatography. Since then, many efforts have been made by Hill's group and several other groups to develop ion mobility spectrometers for GC [35–38], SFC [39–47], HPLC [48] and unified chromatography [49,50]. IMS can be recognized as a type of time-of-flight mass spectrometer which performs at atmospheric pressure or as an electron-capture detector to which an ion separation chamber has been added (Fig. 8). Ions generated from the analytes migrate under the influence of an electric field against a counter-current flow of gas. The ions are recorded
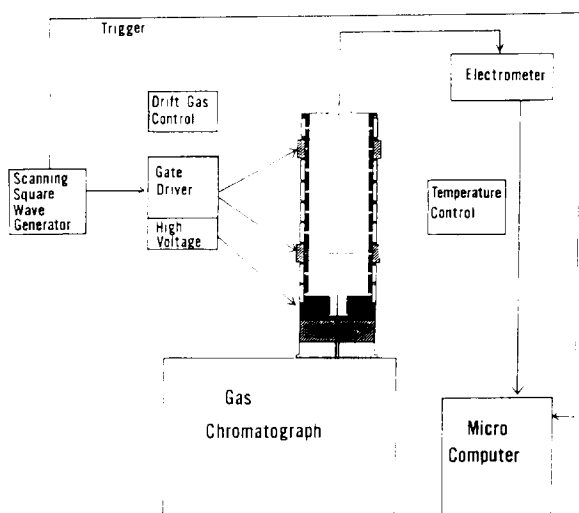

Fig. 8. Diagram of an ion mobility detector. From Ref. [50]; © J. High Resolut. Chromatogr.

when they arrival at the end of the migration space. Different ionization means have been investigated. [63]Ni foil is commonly used as a photoionization source [35,42,44] for IMS.

Photoionization accomplished through the use of a short-wavelength UV lamp [36] and a laser source [37] has also been investigated. IMS as a method for HPLC detection has not been applied as successfully as in GC and SFC. Initial attempts to interface electrospray ionization into ion mobility spectrometry (IMS) were reported by Gieniec et al. [51]. Further investigations of nebulization and ionization using electrospray has been investigated by Hill's group and promising results have been obtained [48–50,52]. Stastna et al. [53] described and evaluated a simple detection technique using electrospray ionization and a copper plate electrode. Fourier transform IMS was developed with an improvement of signal/noise values [54,55] and has been applied for a wide variety of sample types [56].

Most modified CO_2 mobile phases have little effect during SFC analyses [46,47] due to the ability of the IMS to tolerate an organic solvent in the mobile phase. Hill and McMinn [57] have applied IMS to the detection after open tubular CG separation of silylated monosaccharides cleaved from Sertoli cell glycoprotein, after SFC with CO_2 as the mobile phase for the detection of polydimethylsilicone oligomers, and after LC with methanol–water (20:80) as mobile phase.

3.3. Evaporative light-scattering detection

Universal detection with evaporative light-scattering detection (ELSD) has become a popular detection means for analytes which lack a UV chromophore because FID cannot be used with most organic liquid mobile phases. The performance of ELSD as a detection method for both HPLC and packed-column SFC analyses have been investigated [58,59]. The combination of packed capillary columns and ELSD under SFC conditions was demonstrated by Hoffmann and Greibrokk [60] in 1989. Demirbuker et al. [61] constructed in 1993 a miniaturized ELSD system for packed capillary column SFC. The disadvantage of ELSD is that it cannot be applied to most

GC analyses due to the low sensitivity to low-molecular-mass compounds.

3.4. Ultraviolet absorbance detection

Ultraviolet (UV) absorbance detection has been widely used in HPLC analysis and is also the most common detection method in packed-column SFC using organic or modified mobile phases. In order to apply UV detection in open tubular column SFC, Hirata and Katoh [62] designed a temperature and pressure-controlled UV cell; this allowed the use of UV detection during pressure programming without baseline drift. Since the 1960s, UV absorbance detection has been applied to GC in several forms [63–67], but no commercial UV detector which implements absorbance monitoring in capillary GC is available. Bornhop and Wangsgaard [68] described the use of capillary GC analysis and on-the-fly spectral scanning for the identification of two isomeric chlorobenzenes. They also indicated that GC–UV is a non-destructive, information-rich technique and should show analytical utility, especially in combination with FID or MS. Packed capillary column SFC and HPLC with a Z-configuration capillary UV detector can provide high-sensitivity analysis [31] because of the concentrated solutes in the column due to the small column internal diameter and the long path length (22 mm for Kontron UV detector) of Z-configuration capillary UV detectors [28].

3.5. Plasma-based detection

Uden [69] has reviewed the use of plasma atomic emission spectroscopy-based detectors in chromatography. Such element-selective detectors are much more common in GC than in LC or SFC. Jin et al. [70] demonstrated the utility of the microwave plasma torch for GC and SFC detection. An investigation of Cl-specific detection for open-tubular SFC was made by Zhang et al. [71] by using a He microwave-induced plasma. Jinno and co-workers [72,73] investigated the inductively coupled plasma (ICP) as a detector for packed-microcolumn SFC and found that the ICP was not compatible with the effluent

flow-rate of conventional packed columns, but packed capillary columns worked well [73]. Forbes et al. [74] also demonstrated the use of an ICP for detection in open tubular column SFC and showed the selective detection of Si-containing compounds.

3.6. Fourier transform infrared detection

FT-IR detection for SFC has been reviewed [75–78], while Norton et al. [78] proposed *A unified approach to the chromatography–FT-IR interface*. They described a direct-deposition interface (deposition onto a moving plate with mobile phase elimination) which is compatible with GC, LC and SFC. Griffiths et al. [79] also demonstrated a unified approach for coupling open tubular GC, LC and SFC with off-line FT-IR micro-spectroscopy using solvent elimination by depositing the analyte in a suitable small spot while removing the mobile phase. Raynor et al. [80] and Healy et al. [81] demonstrated the use of supercritical Xe as mobile phase in the on-line approach, especially useful since it is IR transparent, and high sensitivity was achieved. Shah et al. [82] investigated the use of the on-line approach to compare SFC and HPLC separations of steroids. Raynor et al. [83] investigated the performance of a solvent-elimination interface for open tubular SFC–FT-IR. They also described a low-volume flow cell designed for open tubular SFC–FT-IR–FID taking advantage of the non-destructive nature of FT-IR detection and the resolving power of open tubular columns [84]. Jinno and Fujimoto [85] described an interface for off-line, solvent-elimination FT-IR detection after microcolumn LC or SFC separation.

3.7. Mass spectrometric detection

MS is probably the most powerful detection method in analytical chemistry, and can be coupled to GC, SFC and HPLC by means of different types of interface. A unified chromatograph coupled with a mass spectrometer is a promising technique because such a system is very flexible and more effective for the analyses

of widely varying samples. Packed capillary columns are superior to conventional packed columns in the cases of HPLC–MS and SFC–MS. Better quantitative reproducibility and higher sensitivity can be achieved because of splitless coupling and the higher concentration of solutes in the columns. For unified chromatography–MS, a special interface is required which is suited to either GC, SFC or LC or can be simply transformed from one mode to another.

Recently, many efforts have been made to improve such interfaces especially for HPLC and SFC detection. Development of flexible (unified) interfaces for detection following different separation modes have been carried out. Hawthorne and Miller [86] constructed an open tubular direct interface which was then applied to analysis of polycyclic aromatic hydrocarbons with molecular masses up to 532. This system could be converted from SFC–MS to GC–MS mode in about 20 min [86]. Sanders et al. [87] investigated a particle-beam interface and reviewed the potential utility of this interface for LC–MS and SFC–MS of non-polar, relatively volatile compounds. Kalinoski and Hargiss [88] demonstrated a removable probe interface for direct-fluid-introduction SFC–MS. The interface allowed the conversion to GC–MS mode in several minutes without venting the mass spectrometer. Olesik [89] reviewed SFC–MS interface designs as well as the key control variables. Matsumoto et al. [90] described an atmospheric pressure chemical ionization interfacing method for packed capillary column SFC using a nebulizing interface originally developed for LC–MS and optimized and evaluated the interface using a variety of oligomeric and non-polar analytes.

4. Applications of unified chromatography

In recent investigations of unified chromatography, two or three separation modes (GC, SFC or LC) have been carried out with one system and sequential GC–SFC analyses are commonly demonstrated. In general, applications of unified chromatography can cover all areas that GC, SFC and LC have covered, although almost all

recent investigations have been carried out on capillary columns (open tubular or packed capillary). The recent targets of unified chromatographic analysis are focused on increasing resolution and separation range.

The first application of sequential GC–SFC analysis in a single chromatographic run was demonstrated by Ishii et al. [11], the GC separation of aromatic hydrocarbons followed by SFC separation of styrene oligomers (Fig. 9) with diethyl ether vapour as the mobile phase.

Takeuchi and co-workers used 0.5–0.7 mm I.D. glass-lined stainless-steel columns packed with 5-μm Develosil ODS-5 particles with methanol or diethyl ether as mobile phases under SFC and/or LC conditions (depends on the temperature and pressure in the column) to separate styrene oligomers, non-ionic detergents
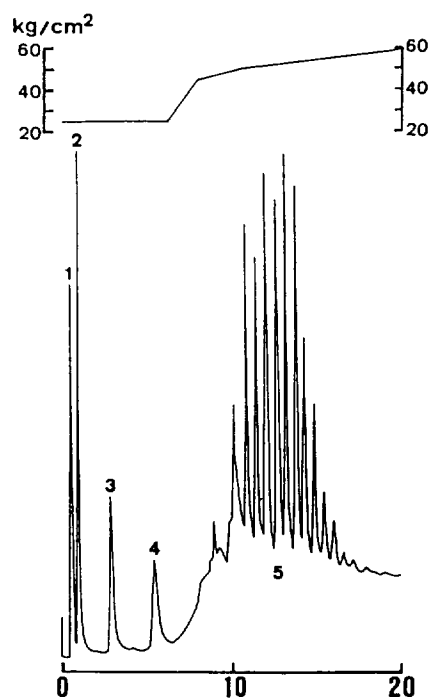

Fig. 9. Chromatogram of a sequential GC and SFC separation of a mixture of aromatic hydrocarbons and styrene oligomers on a 15 m × 0.5 mm I.D. column packed with 5-μm Develosil 100-5 particles. Peaks: 1 = benzene; 2 = naphthalene; 3 = anthracene; 4 = pyrene; 5 = polystyrene A-1000. From Ref. [11]; © *J. High Resolut. Chromatogr. Chromatogr. Commun.*

and methylphenylsiloxane oligomers [12]. Later they employed open tubular columns to analyse dialkyl phthalates and a surfactant using carbon dioxide as mobile phase with positive temperature programming or negative temperature programming to achieve LC conditions [13].

Takeuchi and co-workers also investigated the use of packed capillary columns for GC analysis; either the vapour of organic substances or carbon dioxide was employed as carrier gas [17,18]. They separated a mixture of alkylbenzenes with

a 30 cm × 0.5 mm I.D. glass-lined capillary columns packed with 10-μm Develosil 60-10 particles. Hexane or diethyl ether vapour was used as mobile phase in these analyses [17]. They also used carbon dioxide as mobile phase to separate a light oil with carbon number 9 to 24 and a kerosine with carbon number 8 to 15 under GC conditions with 30 cm × 0.3 mm I.D. glass-lined capillary columns packed with 5-μm Capcell Pack C_{18} [18].
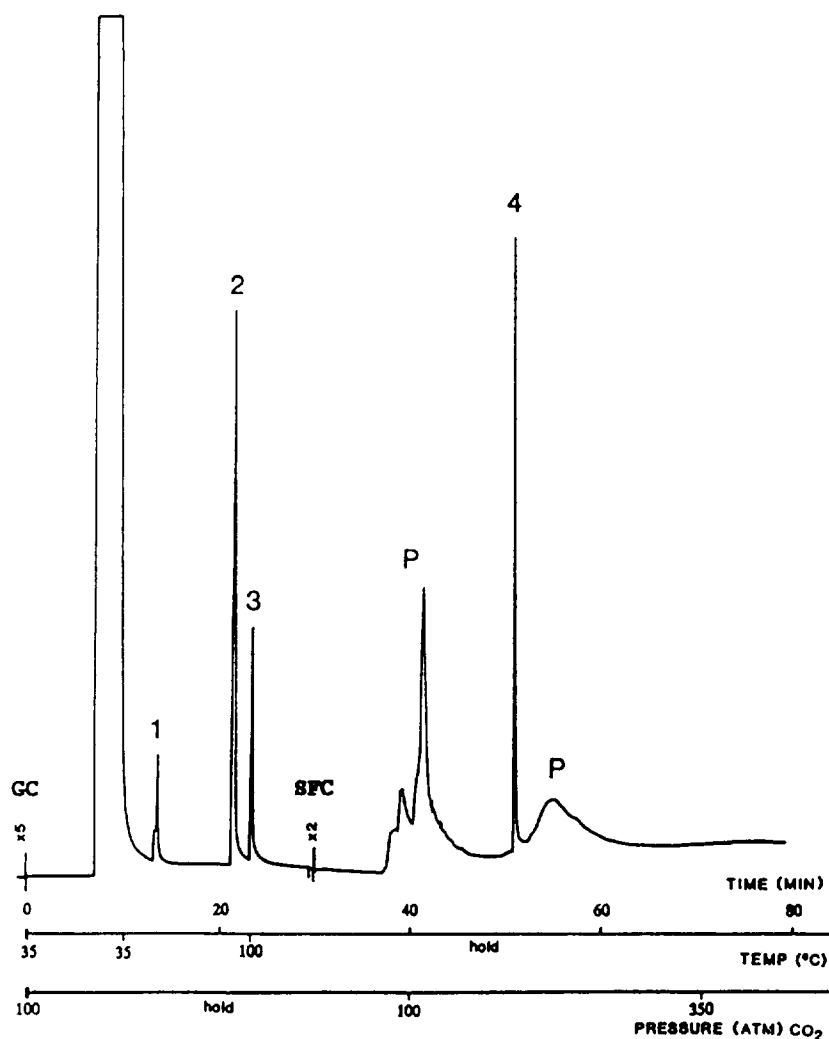
Gemmel et al. applied an isocratic program-

Fig. 10. Chromatogram of a sequential GC and SFC analysis of a nail lacquer sample on a 10 m × 100 μm open tubular fused-silica column coated with 0.25-μm SB-Methyl-100 stationary phase. Peaks: 1 = ethyl acetate; 2 = butyl acetate; 3 = toluene; 4 = dibutyl phthalate; P = polymers. From Ref. [23]; © *Anal. Chem.*

ming sequence from GC to SFC, or from GC, via SFC to LC using consecutive gradients to separate oligomeric species [20] and a mixture of aromatic hydrocarbons and styrene oligomers [21] by changing the oven temperature and operating pressure with pentane or dioxane as mobile phases.

Steenackers and Sandra [22] analyzed mixtures of C_5–C_{16} hydrocarbons with carbon dioxide as mobile phase under both LC and SFC conditions by changing the operating pressure and temperature. A 51.5 cm × 100 μm I.D. fused-silica column packed with 5-μm Spherisorb ODS-2 was employed in the analysis.

Davies and Yang [23] demonstrated the applications of sequential GC and SFC separation for the analysis of a mixture of gasoline and crude oil and a sample of clear nail lacquer (Fig. 10) with 100 μm I.D. open tubular columns. The GC section was carried out with helium at high pressures (158 atm) with temperature programming and the SFC section was performed with

supercritical fluid carbon dioxide with pressure programming and a constant temperature.

Liu and Yang [24,25] demonstrated the separations of a synthetic n-alkane mixture, a synthetic alcohol mixture, a mixture of unleaded gasoline and crude oil, and a mixture of a synthetic alcohols and fatty acids by sequential GC–SFC analyses [24,25]. Capillary columns packed with polymer stationary phase and high-pressure helium and carbon dioxide mobile phases were employed.

Robinson et al. [26] applied an independent optimization GC–SFC system to analyse a mixture containing n-alkanes and synthetic triglycerides, a light lube stock contaminated by motor gasoline, a synthetic mixture of selected organic compounds in purified coconut and soybean oil, and a commercial decongestant. Higher resolution in the sequential GC–SFC analyses was achieved due to the optimized operating conditions in both GC and SFC separations. Later, Tong et al. [27] applied 50 μm I.D. open
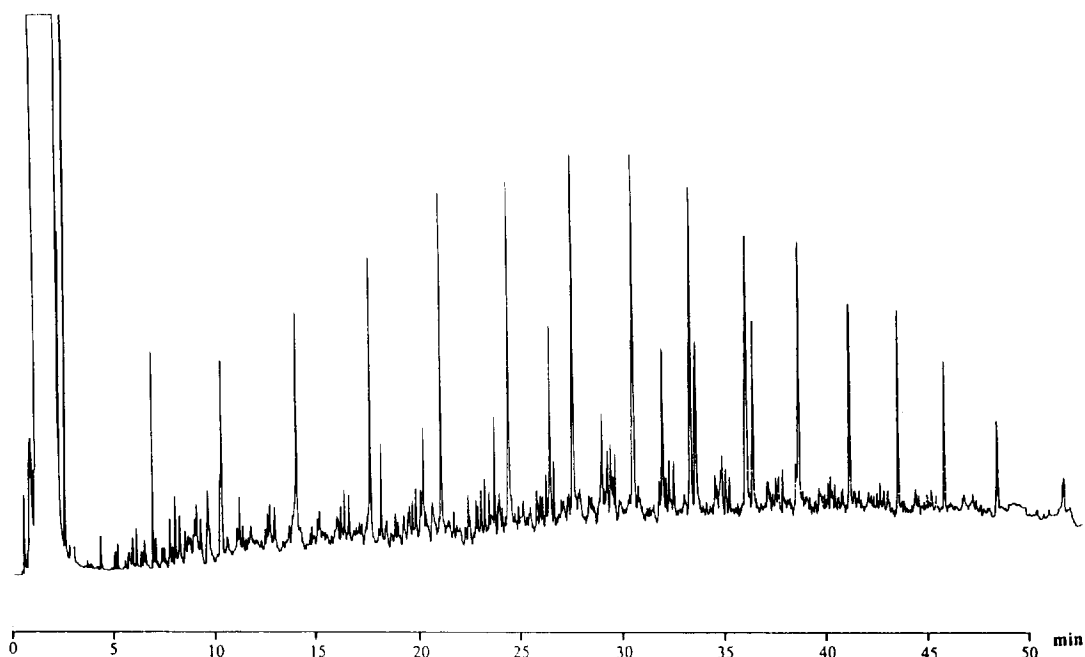

Fig. 11. GC chromatogram of an analysis of a dichloromethane solution of a diesel fuel. Conditions: valve injection with 60-nl injection volume, 10 m × 50 μm I.D. coated with a 0.25-μm film of SB-Methyl fused-silica open tubular column, helium mobile phase at 12 atm, FID, and temperature programming from 60 to 240°C at 4°C/min.

tubular columns to the sequential GC–SFC analyses of gasoline contaminated heavy lubricating oil and a commercial household wax preparation based on beeswax and obtained further improvements of GC and SFC resolutions.

Tong et al. [29] also applied the unified chromatography to petrochemical analysis with the independently optimized GC, SFC and HPLC system. They separated samples of diesel in lube oil and deasphalted Arabian heavy crude oil by means of sequential GC–SFC analysis. SFC analyses of a sample of diesel fuel with either UV detection or FID were performed on a packed capillary column. They demonstrated the considerable advantage of sequential GC–SFC for the analysis of mixtures of components with a wide range of volatilities.

High-resolution GC, SFC, micro-LC and sequential GC–SFC analyses were achieved by using the improved unified system [30] and newly developed packed capillary columns [31]. Tong et al. separated diesel fuel with valve injection using a 50 μm I.D. SB-Methyl fused-silica open tubular column under GC conditions. Fig. 11 shows that high resolution and fast separation

were achieved due to the high efficiency of the columns. This procedure avoids the problems of quantitative reproducibility for a wide-volatility-range sample inherent in the split injection technique, and the operating inconveniences with normal splitless injection techniques. SFC analyses were carried out on both open tubular columns and packed capillary columns. The analysis of an ethoxylate on a open tubular column was performed with pressure programming from 50 to 415 atm and temperature programming from 80 to 240°C. High-resolution SFC analyses of crude oil (Fig. 12) were obtained with a 50 cm packed capillary column. They showed micro-LC application for the separation of nitrated polycyclic aromatic hydrocarbons (Fig. 13) and purity analysis using packed capillary columns [30]. They improved the se-
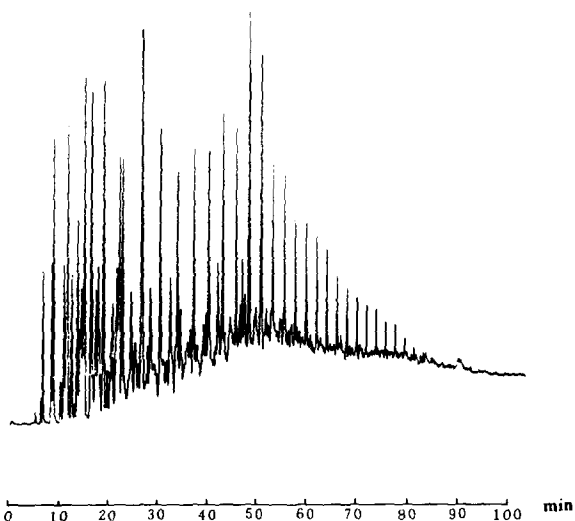

Fig. 12. SFC chromatogram of crude oil. Conditions: 50 cm × 0.25 mm I.D. fused-silica column packed with 5-μm ODS-1 particles; FID; carbon dioxide mobile phase at 100°C, from 100 atm (hold 5 min) to 415 atm at 2.5 atm/min.
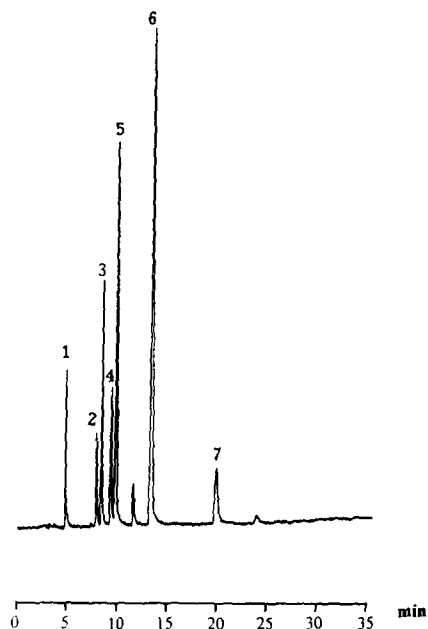

Fig. 13. HPLC chromatogram for an isocratic separation of an acetonitrile solution of nitrated polycyclic aromatic hydrocarbons. Conditions: 40 cm × 0.25 mm I.D. fused-silica column packed with 5-μm ODS-1 particles; UV detection at 254 nm; acetonitrile–water–methanol (60:30:10, v/v) mobile phase at room temperature. Peaks: 1 = 4-nitroaniline; 2 = 1-nitronaphthalene; 3 = 2-nitronaphthalene; 4 = 2-nitrofluorene; 5 = 3-nitrobiphenyl; 6 = 9-nitroanthracene; 7 = 1-nitropyrene.
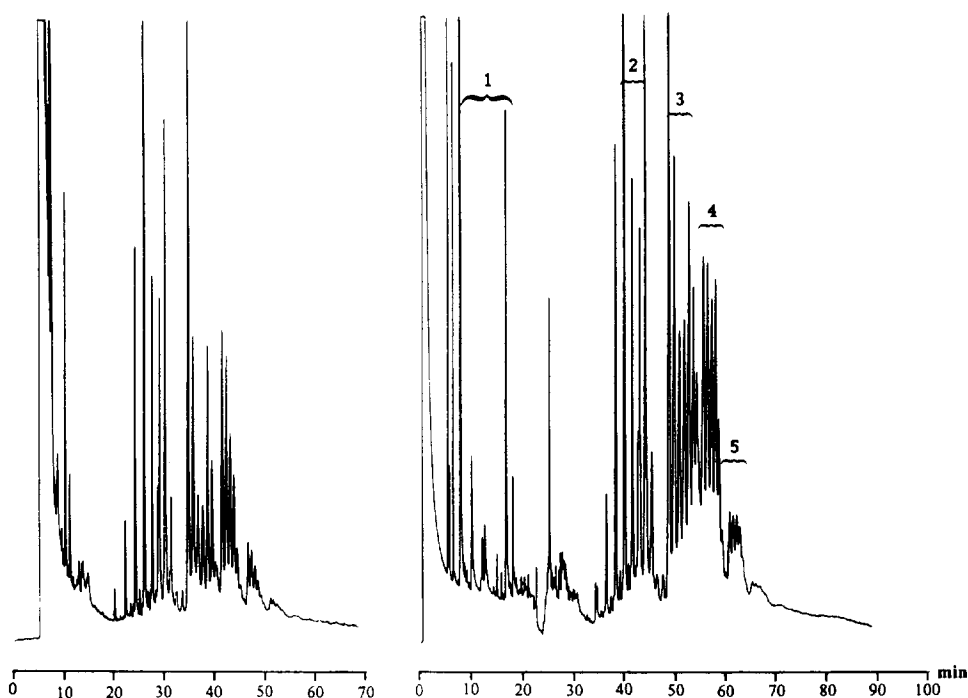
Fig. 14. Separations of a dichloromethane solution of a household wax. Conditions: 10 m × 50 μm I.D. coated with a 0.25-μm film of SB-Methyl fused-silica open tubular column. Valve injection and FID. (Left) Carbon dioxide mobile phase, at 120°C, from 70 to 360 atm at 5 atm/min. (Right) Helium mobile phase at 15 atm from 40 to 150°C at 5°C/min, and then to 120°C within 1 min; carbon dioxide mobile phase at 120°C from 120 to 360 atm at 5 atm/min. Peaks: 1 = terpenes; 2 = C_{23}–C_{35} alkanes; 3 = $C_{15}H_{31}$COOR esters. R = $C_{24}H_{49}$ to $C_{34}H_{69}$; 4 = diesters $C_{15}H_{31}$COOC$_{15}H_{30}$COOR. R = $C_{24}H_{49}$ to $C_{34}H_{69}$; 5 = triesters.

quential GC–SFC analyses for the separations of the samples of household wax (Fig. 14) and pesticide-contaminated vegetable oil (Fig. 15) using 50 μm I.D. open tubular columns. The volatile components were firstly analysed by GC with temperature programming and then the non-volatile components were eluted by SFC with pressure programming. Both separations in either GC or SFC mode were performed under optimum conditions. Due to the improved system and the optimization of operating conditions during mobile phase transformation from GC to SFC, there was no loss of resolution in the SFC separations.

5. Future of unified chromatography

The use of the selectivity of LC and the detection capability with FID, TID, electron-

capture detection and FPD under GC or SFC conditions in a sequential LC–GC or LC–SFC analysis can be applied to the samples which lack a chromophore and are difficult to separate. Supercritical fluid modified with an organic solvent as mobile phase with composition gradient and temperature programming (sequential SFC–LC) on packed capillary columns is also a promising technique to solve the problems of complex sample analysis.

A unified chromatograph coupled with a mass spectrometer should become a very useful technique due to its flexibility. However, a special interface is required, which is suited to either GC, SFC or LC, and which can be transformed from one mode to another by simply changing parts.

The development of a new detection method which is applicable in all separation modes is very important for the achievement of a com-
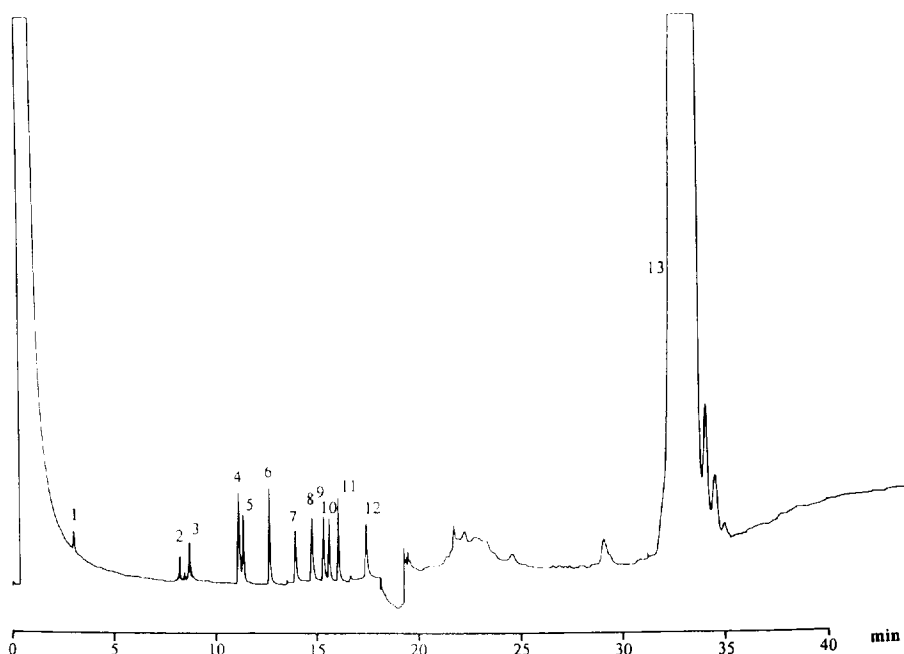
Fig. 15. GC–SFC chromatogram of pesticides spiked into vegetable oil. Conditions: 10 m × 50 μm I.D. coated with a 0.25-μm film of SB-Methyl fused-silica open tubular column; FID: helium mobile phase at 12 atm, temperature programming from 150 to 248°C at 5°C/min and then to 100°C within 1.5 min; carbon dioxide mobile phase: at 100°C, 200 to 300 atm at 5.5 atm/min. Peaks: 1 = hexachlorocyclopentadiene; 2 = atrazine; 3 = hexachlorobenzene; 4 = alachlor; 5 = heptachlor; 6 = aldrin; 7 = heptachlor epoxide; 8 = γ-chlordane; 9 = trans-nonachlor; 10 = dieldrin; 11 = endrin; 12 = methoxychlor; 13 = vegetable oil triglycerides.

mercial unified chromatograph. A new valve for mode selection, which has smaller dead volume and is more chemically inert is also desirable. New analytical methods and applications are critical in the further development of unified chromatography.

6. Conclusions

As far as both chromatographic instrumentation and analysis are concerned, unified chromatography is a promising technique. Different separation modes can be performed with a single injector and single column and can be carried out in series in a single run. So far, the major difficulty for an effective unified chromatograph is the lack of a unique detector or interface which is suited to all separation modes.

The advantages of unified chromatography are as follows: (1) the unification and miniaturisation

of chromatographic instruments; (2) the possibility of analysis of a variety of samples with a single chromatographic instrument in which all separation modes can be selected; (3) the use of a single separation run for samples with a wide range of volatility, in which two or three separation operations are combined; (4) injection without any limitations imposed by the physical state of the mobile phase; (5) the combination of the advantages of different chromatographic modes, for samples which cannot be analysed in a single mode.

In general, unified chromatography may increase peak capacity, injection volume, separation range and selectivity, and avoid detection limitations; it can also achieve sequential separations with a single system for samples which cannot be separated in a single chromatographic run. A unified chromatograph can save laboratory space, and is flexible and convenient for analytical method development.

References

[1] J.C. Giddings, Dynamics of Chromatography, Marcel Dekker, New York, 1965.

[2] J.C. Giddings, Unified Separation Science, Wiley, 1991.

[3] D.E. Martire J. Chromatogr., 452 (1988) 17.

[4] D.E. Martire J. Liq. Chromatogr., 11 (1988) 1779.

[5] D.E. Martire J. Chromatogr., 461 (1989) 165.

[6] M.L. Lee and K.E. Markides, in Analytical Supercritical Fluid Chromatography and Extraction, Chromatography Conferences, Provo, UT, 1990.

[7] F.J. Yang (Editor), Microbore Column Chromatography: A Unified Approach to Chromatography, Marcel Dekker, New York, 1989.

[8] D. Ishii and T. Takeuchi, J. Chromatogr. Sci., 27 (1989) 71.

[9] S.L. Pentoney, Jr., A. Giorgetti and P.R. Griffiths, J. Chromatogr. Sci., 25 (1987) 93.

[10] K.D. Bartle, I.L. Davies, M.W. Raynor, A.A. Clifford and J.P. Kithinji, J. Microcolumn Sep., 1 (1989) 63.

[11] D. Ishii, T. Niwa, K. Ohta and T. Takeuchi, J. High Resolut. Chromatogr. Chromatogr. Commun., 11 (1988) 800.

[12] T. Takeuchi, T. Niwa and D. Ishii, Chromatographia, 23 (1987) 929.

[13] T. Takeuchi, K. Ohta and D. Ishii, Chromatographia, 25 (1988) 125.

[14] D. Ishii, T. Takeuchi, M. Saito and H.H. Toshinobu, Japan Pat., JP 88265164 A2 (1988).

[15] P. Lu, L. Zhou, C. Wang, G. Wang, A. Xia and F. Xu, J. Chromatogr., 186 (1979) 25.

[16] D. Tong, F. Xu and P. Lu, Chromatographia, 23 (1987) 499.

[17] T. Takeuchi, T. Hamanaka and D. Ishii, Chromatographia, 25 (1988) 993.

[18] T. Takeuchi, K. Ohta and D. Ishii, Chromatographia, 27 (1989) 182.

[19] T. Takeuchi, Y. Hashimoto and D. Ishii, J. Chromatogr., 402 (1987) 328.

[20] B. Gemmel, F. Schmitz and E. Klesper, J. Chromatogr., 455 (1988) 17.

[21] B. Gemmel, F.P. Schmitz and E. Klesper, J. High Resolut. Chromatogr. Chromatogr. Commun., 11 (1988) 901.

[22] D. Steenackers and P. Sandra, J. High Resolut. Chromatogr., 14 (1991) 842.

[23] I.L. Davies and F.J. Yang, Anal. Chem., 63 (1991) 1242.

[24] Y. Liu and F.J. Yang, Anal. Chem., 63 (1991) 926.

[25] Y. Liu and F.J. Yang, Anal. Chem., 63 (1994) 1344.

[26] R.E. Robinson, D. Tong, R. Moulder, K.D. Bartle and A.A. Clifford, J. Microcol. Sep., 3 (1991) 403.

[27] D. Tong, K.D. Bartle and A.A. Clifford, J. High Resolut. Chromatogr., 15 (1992) 505.

[28] D. Tong and K.D. Bartle, J. Microcol. Sep., 5 (1993) 237.

[29] D. Tong, K.D. Bartle and R.E. Robinson, J. Chromatogr. Sci., 31 (1993) 77.

[30] D. Tong, K.D. Bartle, A.A. Clifford and R.E. Robinson, Analyst, submitted for publication.

[31] D. Tong, K.D. Bartle and A.A. Clifford, J. Microcol. Sep., 6 (1994) 249.

[32] V.L. McGuffin and M. Novotny, Anal. Chem., 55 (1983) 2296.

[33] M.V. Novotny and D. Ishii (Editors), Microcolumn Separations (Journal of Chromatography Library, Vol. 30), Elsevier, Amsterdam, 1985.

[34] H.H. Hill, presented at the 25th Annual Meeting of the Liquid Chromatographic Discussion Group of Japan, Kyoto, February 1984.

[35] M.A. Baim and H.H. Hill, Jr., Anal. Chem., 54 (1982) 38.

[36] M.A. Baim, R.L. Eatherton and H.H. Hill, Jr., Anal. Chem., 55 (1983) 1761.

[37] D.M. Lubman and H.H. Hill, Jr., Anal. Chem., 55 (1983) 867.

[38] S. Rokushika, H. Hatano and H.H. Hill, Jr., Anal. Chem., 58 (1986) 361.

[39] R.L. Eatherton, M.A. Morrissey, W.F. Siems and H.H. Hill, Jr., J. High Resolut. Chromatogr. Chromatogr. Commun., 9 (1986) 154.

[40] S. Rokushika, H. Hatano and H.H. Hill, Jr., Anal. Chem., 59 (1987) 8.

[41] R.L. Eatherton, M.A. Morrissey and H.H. Hill, Jr., Anal. Chem., 60 (1988) 2240.

[42] S. Rokushika, H. Hatano and H.H. Hill, Jr., Anal. Chem., 61 (1989) 601.

[43] R.H. St. Louis and H.H. Hill, Jr., J. High Resolut. Chromatogr., 13 (1990) 628.

[44] C.B. Shumaten and H.H. Hill, Jr., J. Chromatogr., 505 (1991) 215.

[45] M.A. Morrissey and H.M. Widmer, J. Chromatogr., 552 (1991) 551.

[46] M.X. Huang, K.E. Markides and M.L. Lee, Chromatographia, 31 (1991) 163.

[47] H.H. Hill, Jr., R.H. St. Louis, M.A. Morrissey, C.B. Shumate, W.F. Siems and D.C. McMinn, J. High Resolut. Chromatogr., 15 (1992) 417.

[48] D.G. McMinn, J.A. Kinzer, and C.B. Shumate, W.F. Siems and H.H. Hill, Jr., J. Microcol. Sep., 2 (1990) 188.

[49] H.H. Hill, R.H. St. Louis, M.A. Morrissey, C.B. Shumate and D.G. McMinn, 12th Int. Symposium on Capillary Chromatography, Kobe, 11–14 September, 1990, p. 864.

[50] H.H. Hill, R.H. St. Louis, M.A. Morrissey, C.B. Shumate, W.F. Siems and D.G. McMinn, J. High Resolut. Chromatogr., 15 (1992) 417.

[51] J. Gieniec, H.L. Cox, Jr., D. Teer and M. Dole, presented at the 20th Annual Conference on Mass Spectrometry and Allied Topics, 1972, abstracts, p. 267.

[52] Y.H. Chen and H.H. Hill, Jr., J. Microcol. Sep., 6 (1994) 515.

[53] M. Stastna, M. Krejci and V. Kahle, J. Microcol. Sep., 6 (1994) 435.

[54] F.J. Knorr, R.L. Eatherton, W.F. Siems and H.H. Hill, Jr., Anal. Chem., 57 (1985) 402.

[55] R.H. St. Louis, W.F. Siems and H.H. Hill, Jr., *Anal. Chem.*, 64 (1992) 171.

[56] H.H. Hill and E.E. Tarver, in K. Jinno (Editor), *Hyphenated Techniques in Supercritical Fluid Chromatography and Extraction (Journal of Chromatography Library*, Vol. 53), Elsevier, Amsterdam, 1992, p. 9.

[57] H.H. Hill and D.G. McMinn (Editors), *Detectors for Capillary Chromatography*, Wiley, New York, 1992.

[58] M. Dreux and M. Lafosse, *Analusis*, 20 (1992) 587.

[59] M. Lafosse, C. Elfakir, L. Morin-Allory and M. Dreux, *J. High Resolut. Chromatogr.*, 15 (1992) 312.

[60] S. Hoffmann and T. Greibrokk, *J. Microcolumn Sep.*, 1 (1989) 35.

[61] M. Demirbuker, P.E. Anderson and L.G. Blomberg, *J. Microcolumn Sep.*, 5 (1993) 141.

[62] Y. Hirata and S. Katoh, *J. Microcolumn Sep.*, 4 (1992) 503.

[63] W. Kaye, *Anal. Chem.*, 34 (1962) 287.

[64] M. Novotny, F.J. Schwende, M.J. Hartigan and J.E. Purcell, *Anal. Chem.*, 52 (1980) 736.

[65] M. Kube, M. Tierney and D.M. Lubman, *Anal. Chim. Acta*, 171 (1985) 373.

[66] A.K. Adams, D.L. Van Engelen and L. Thomas, *J. Chromatogr.*, 303 (1984) 341.

[67] V. Lagesson and J.M. Newman, *Anal. Chem.*, 61 (1989) 1249.

[68] D.J. Bornhop and J.G. Wangsgaard, *J. High Resolut. Chromatogr.*, 14 (1991) 344.

[69] P.C. Uden, *Anal. Appl. Spectrosc. 2 [Proc. Int. Conf.]*, 1990, (1991) 165.

[70] Q. Jin, F. Wang, C. Zhu, D.M. Chambers and G.M. Hieftje, *J. Anal. At. Spectrom.*, 5 (1990) 487.

[71] L. Zhang, J.W. Carnahan, R.E. Winans and P.H. Neill, *Anal. Chem.*, 63 (1991) 212.

[72] K. Jinno, H. Mae and C. Fujimoto, *J. High Resolut. Chromatogr.*, 13 (1990) 13.

[73] C. Fujimoto, H. Yoshida and K. Jinno, *J. Microcolumn Sep.*, 2 (1990) 146.

[74] K.A. Forbes, J.F. Vecchiarelli, P.C. Uden and R.M. Barnes, *Anal. Chem.*, 62 (1990) 2033.

[75] D. Wieboldt, G. Adams and J.M. Bruna, *Tec. Lab.*, 12 (1989) 386.

[76] K.D. Bartle, M.W. Raynor, A.A. Clifford, I.L. Davies, J.P. Kithinji, G.F. Shilstone, J.M. Chalmers and B.W. Cook, *J. Chromatogr. Sci.*, 27 (1989) 283.

[77] B. Beccard and O. Barres, *Spectra 2000 [Deux Mille]*, 145 (1990) 53.

[78] K.L. Norton, A.J. Lange and P.R. Griffiths, *J. High Resolut. Chromatogr.*, 14 (1991) 225.

[79] P.R. Griffiths, A.M. Haefner, K.L. Norton, D.J.J. Fraser, D. Pyo and H. Makishima, *J. High Resolut. Chromatogr.*, 12 (1989) 119.

[80] M.W. Raynor, G.F. Shilstone, K.D. Bartle, A.A. Clifford, M. Cleary and B.W. Cook, *J. High Resolut. Chromatogr.*, 12 (1989) 300.

[81] M.A. Healy, T.J. Jenkins and M. Poliakoff, *Trends Anal. Chem.*, 10 (1991) 92.

[82] S. Shah, M.A. Khorassani and L.T. Taylor, *Chromatographia*, 27 (1989) 441.

[83] M.W. Raynor, K.D. Bartle, A.A. Clifford, J.M. Chalmers, T. Katase, C.A. Rouse, K.E. Markides and M.L. Lee, *J. Chromatogr.*, 505 (1990) 179.

[84] M.W. Raynor, G.F. Shilstone, K.D. Bartle, A.A. Clifford, M. Cleary and B.M. Cook, *J. Microcol. Sep.*, 1 (1989) 101.

[85] K. Jinno and C. Fujimoto, *Prog. HPLC*, 4 (1989) 273.

[86] S.B. Hawthorne and D.J. Miller, *J. Chromatogr.*, 468 (1989) 115.

[87] P.E. Sanders, E. Sheehan, J. Buchner, R. Willoughby, M. Dilts, T. Marecic and J. Dulak, in W.G. Jennings and J.G. Nikelly (Editors), *Capillary Chromatography*, Hüthig, Heidelberg, 1991, p. 131.

[88] H.T. Kalinoski and L.O. Hargiss, *J. Chromatogr.*, 474 (1989) 69.

[89] S.V. Olesik, *J. High Resolut. Chromatogr.*, 14 (1991) 5.

[90] K. Matsumoto, S. Nagata, H. Hattori and S. Tsuge, *J. Chromatogr.*, 605 (1992) 87.