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# Review

# Developments in multidimensional separation systems

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ABSTRACT

Coupled-column systems in a multidimensional mode are increasingly used in order to obtain greater selectivity and sensitivity for the determination of trace components in complex matrices, and to increase the information content of an analysis in the characterization of complex samples. A review of the various strategies used in chromatography to couple orthogonal separation stages is presented, with emphasis on instrumental design and the role of miniaturization.

CONTENTS



## 1. INTRODUCTION

Single-stage (linear) chromatographic systems offer high resolving power, which is essential for the analysis of complex samples and the determination of trace-level impurities in a wide variety of complex matrices. With efforts placed in obtaining lower limits of detection in increasingly complex samples, stringent demands are being placed on the resolving power of chromatographic systems. Increases in resolution can be achieved by variations in the plate number, $N$, selectivity, $\alpha$, or capacity factor, $k'$; however, adjustment of the capacity factor has a limited influence on resolution, (and only at low values); large numbers of theoretical plates can be realized using the technology available, but because resolution does not increase as greatly by the generation of further plates, increasing column

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length often yields marginal increases in resolution with the corresponding increase of analysis time to unacceptable levels. Since selectivity has the greatest influence on resolution, it is therefore the variable that attracts the most attention.

The limitations of single stage separation systems have been recognized for many years, and in order to describe separations of a multicomponent mixture, Giddings introduced the concept of peak capacity [1], which is defined as the maximum number of components, $n$, that can be placed side by side in the available separation space with a given resolution which satisfies the analytical goals [2], and is given by the following equation for unit resolution:

$$n = (1 + N^{1/2}/r) \ln (1 + k'_i) \quad (1)$$

where $r$ = the number of standard deviations taken as equaling the peak width (typically 4), and $k'_i$ = the capacity factor of the last peak in a series.

Modern high-resolution chromatographic systems yield peak capacities which are calculated to be in the range of 100–300. These results would appear adequate to resolve components in a mixture where the number of components, $m$, is less than the peak capacity of the system; however, components in a complex mixture are usually not uniformly distributed, and appear randomly, overlapping each other. Giddings and Davis [3] developed the statistical model of overlap to study the seriousness of said component overlap, which becomes apparent when the number of visible peaks, $V$, in a chromatogram is estimated by the following equation:

$$V = me^{-m/n} \quad (2)$$

providing that $n$ is chosen as the value corresponding to a resolution of 0.5. Assuming that the number of components in a mixture can be estimated (and in many cases, this cannot be done), and in the case in which the number of components equals the peak capacity of the system used, the maximum number of visible peaks will be equivalent to 37% of the system's peak capacity. Further, the number of peaks in a chromatogram which represent a single component, $S$, is given by:

$$S = me^{-2m/n} \quad (3)$$

which yields a value of only 18% of the peak capacity, using the conditions described above.

These calculations help us to understand the limitations encountered in the separation of components in a complex matrix, even in cases where single column (linear) chromatographic systems are operated close to the theoretical limits [4].

A practical means of effecting changes in resolution is the introduction of a different fundamental mechanism of interaction by the use of two (or more) separation stages (multidimensional chromatography, or coupled column chromatography). In order to obtain the maximum benefit from multidimensional chromatographic systems, the basic mechanisms controlling separation in each dimension should be different. That is, the system should be non-redundant [5], otherwise, for a column chromatographic system the total peak capacity is given by:

$$n_{tot} = x^{1/2}n \quad (4)$$

where $x$ = number of identical columns used, yielding a coupled system which is only equivalent to a longer linear system. In addition to coupling separation systems with different separation mechanisms, Giddings has suggested the additional requirement that when two components are resolved in the first separation step, they remain resolved throughout the process [3]. A representation of the peak capacity of a planar two-dimensional system is presented in Fig. 1. The maximum peak capacity attainable in a system of this type is given by the product of the peak capacities of each dimension

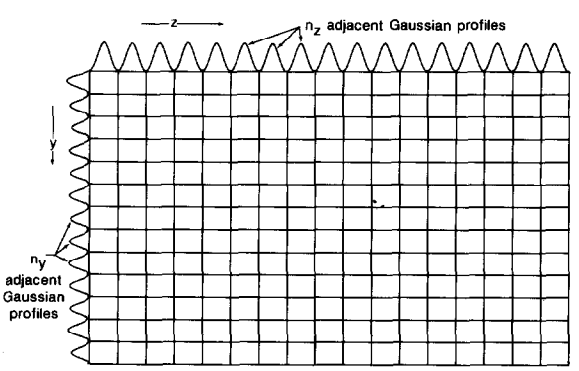


Fig. 1. The peak capacity of a two-dimensional system, represented by the number of boxes is approximately equal to the product of the peak capacities $n_z$ and $n_y$ generated along the two individual axes, as represented by the number of adjacent gaussian profiles [6].

(discounting the additional band broadening of the migrating components in the second dimension [6]). In column chromatography, utilization of the total available separation space would require a large number of secondary columns, so that all the sample cuts taken while eluting in the first dimension could be transferred for subsequent separations. In practice however, only definite fractions of the separation obtained in the first dimension are studied in detail at one time, due in part to the difficulty and awkwardness of a system composed of a multitude of secondary columns. The use of these types of systems is conventionally termed coupled-column chromatography, and their total peak capacities vary according to its design. Total peak capacities can range from $2n$ for two columns of correlated selectivities to a theoretical maximum of $n^2$ for columns of independent selectivities. A representation of the separation space utilized with such a system is presented in Fig. 2.

Multi-stage separations are historically common in the fields of trace analysis, where samples typically contain a large number and variety of components that can potentially interfere with the analytes of interest. Typically, samples are pretreated to reduce the complexity of the original sample by separating some fraction of the matrix from the analytes of interest. Separation schemes for sample pretreatment can include solvent extractions, the use of small packed beds, membranes, or gravity-flow liquid chromatographic columns, for example. Such schemes, where sample preparation and analytical columns are not physically coupled and manual steps are involved, can be grouped under the class of off-line multidimensional separations. In the practice of off-line multidimensional separations, it is recognized that clean-up techniques such as those described above can be time consuming, operator intensive, and can also be difficult to automate and reproduce. Of greater importance in areas of quantitative trace analysis, is the fact that off-line sample pretreatments can be susceptible to solute loss and contamination.

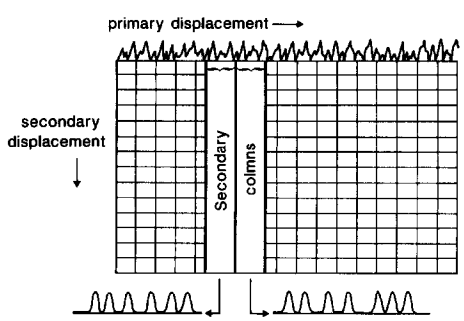


Fig. 2. Superposition of two adjacent secondary columns (represented by wide vertical bars) of a coupled column system on the two-dimensional separation matrix of Fig. 1. The columns are shown diagrammatically with a width proportional to the number of peaks shunted from the primary displacement (column) [6].

An alternative approach to increase in selectivity is the use of several separation stages in an on-line mode, where two (or more) separation systems of relatively high efficiency are coupled together via the use of traps, valves and other means.

## 2. THE ROLE OF MICROCOLUMNS IN MULTIDIMENSIONAL SEPARATIONS

Coupled column systems can utilize combinations of both packed and open tubular chromatographic columns. Coupled column chromatography has been practiced using gas chromatography (GC), supercritical fluid chromatography (SFC), liquid chromatography (LC), and combinations of these, such as LC–GC, LC–SFC, or LC coupled to capillary zone electrophoresis. Supercritical fluid extractions, although not a chromatographic technique in the rigorous sense, has also been used as a preliminary separation stage with other chromatographic systems. Coupled techniques can benefit from miniaturization. The advantages of using capillary columns for GC, for example, are already well documented [7]. In the following sections, a short review of the various separation modes will be discussed. Due to the large volume of information available in the literature regarding coupled column techniques, this review is not intended to be exhaustive, but is intended to point out the major advantages of each separation strategy and summarize the important contributions, with special emphasis on the use of microcolumns.

## 3. MICROCOLUMN LIQUID CHROMATOGRAPHY

Miniaturization of an LC system was initially investigated in the 1970s [8–12] with the recognized benefits of reduced consumption of mobile and sta-

tionary phases, increased mass sensitivity with concentration-sensitive detectors, high separation efficiencies and posibility of new detection techniques [13–15]. The technique has been reviewed extensively [16–19] and the purpose of this section is to detail the salient merits of microcolumn LC as they apply to the on-line coupling to other separation modes.

The reduction of the internal diameter of the LC column utilized for multidimensional chromatographic applications to microcolumn dimensions (< 1.0 mm) introduces various significant advantages to the technique. Elution volumes of microcolumns are more closely matched to capillary column requirements than conventional columns, since successful interfacing requires that broadening of fractions introduced in the second dimension remain minimal. Difficulties in interfacing chromatographic techniques are typically a direct result of the volume and nature of the mobile phase used in the first dimension. The volume of eluent used in microcolumn LC is considerably reduced, which means the solutes of interest are diluted in much less eluent (lower volumetric dispersion). As an example, the peak volume ($V_p$) eluting from an LC column can be calculated by the following equation [17]:

$$V_p = \pi d_c^2 \varepsilon L(1+k')/N^{1/2} \quad (5)$$

where $d_c$ = column diameter, $\varepsilon$ = column porosity and $L$ = column length. The peak volume of a compound eluting from a typical microcolumn of 25 cm × 250 μm I.D., a column porosity of 0.5 and at a $k'$ of 3 with an efficiency of 25 000 plates can be calculated as *ca.* 0.6 μl; a significant reduction when compared to the peak volume given by a conventional column (4.6 mm I.D.) under the same conditions, *ca.* 200 μl. In practice, the peak volumes observed at the detector are somewhat larger, due to the extra-column band broadening contributions of the injector, connections, and detector cell. Nevertheless, this reduction in peak volume can be critical, as it minimizes the problems encountered in the introduction of large volumes of solvent into other separation techniques, such as capillary GC. Because of this peak volume reduction, much larger sections of the LC chromatogram can be introduced into the subsequent separation stages allowing quantitative transfer of the components of interest, resulting in greater reproducibility and better opportunity for quantitative analyses. Microcolums can be effectively prepared at lengths greater than the conventional 25 cm, and greater total column efficiencies can be obtained [20,21].

The column diameter has been shown to have an effect on the efficiency of packed-column systems [22,23]. Fluctuations in the packing density which may occur across the column diameter [24], and temperature gradients generated due to viscous friction [25], may be detrimental contributions which can be minimized by reducing the column diameter. (Heat generated due to viscous friction is expected to be dissipated faster with reduced diameter columns).

The conditions for packing microcolumns for LC reproducibly have been described [26–29]. Typically, columns are packed using a slurry in a suitable solvent and pressures in the range of 400 to 680 atm. Various techniques have been developed to hold the particles in the column and at the same time, minimize flow resistance, such as the use of glass wool [30], wires [31], porous filters [32] and porous polymer discs [33]. In order to simplify the preparation of bed supports for microcolumns, a porous ceramic frit was developed, which we found to yield acceptable performance in terms of ease of preparation and efficiency of the columns used [34].

A perceived limitation of using microcolumns for LC in the context of coupled column separations is the lower sample capacity of the column, which is proportional to the surface area. The effect of sample mass injected on plate height observed at various capacity factors is illustrated in Fig. 3. The results were obtained at linear velocities above the optimum, and therefore the plate heights observed were not as low as could be theoretically or practically obtained. Higher plate heights were observed for components with lower $k'$, which suggests extra-column band broadening, as these effects are expected to be more apparent at lower values of $k'$. Little change was observed for up to 300 ng of material introduced.

The maximum volume that can be injected into a micro LC column has been estimated [17], and is in the range of 60–200 nl for columns dimensions normally used. In practice, high volume loads can be applied if the analytes are dissolved in a weaker solvent than the mobile phase (peak focusing). This procedure allows introduction of relatively large

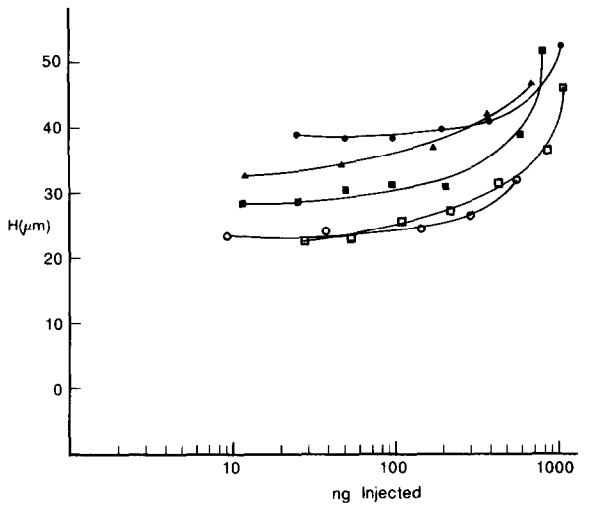


Fig. 3. Plate height ($H$) *vs.* amount injected at various values of $k'$. Column: 12 cm × 250 mm I.D. packed with Zorbax ODS. Eluent: acetonitrile–water (70:30). Flow: 5 ml/min. Injection: 60 nl. Detection: 254 nm. ● = Phenol ($k'$ = 0.48); ▲ = acetophenone ($k'$ = 1.45); ■ = benzophenone ($k'$ = 2.33); ○ = biphenyl ($k'$ = 4.23); □ = dibenzofuran ($k'$ = 4.59).

volumes without excessive loss in efficiency, as shown for microcolumns by Duquet *et al.* [35], who demonstrated that 10-μl volumes could be injected into a microcolumn in this manner without serious loss in efficiency. More recently, introduction of 10 ml into a microcolumn without detrimental effects was demonstrated [36].

In the use of microcolumns for LC in multidimensional separations, the microcolumn is used as a highly efficient pre-separation (clean-up) step or a chemical class fractionation, and therefore, a limited decrease in efficiency due to large injection volumes can be tolerated. Problems of peak band broadening and solute overloading in the LC are seldom critical to the subsequent separation stage. Samples which may contain higher-molecular-mass material, or components which may be irreversibly adsorbed can, as a last resort, be introduced into the LC system. In our experience, when the head of LC the column becomes severely contaminated, removing a short section at the front of the column usually restores the column to nearly its original performance. Another alternative is to backflush the LC column to remove undesirable components prior to subsequent injections.

Various modes of coupled column systems have been developed, the advantages and constraints of which are summarized in the following sections.

## 4. MULTIDIMENSIONAL GAS CHROMATOGRAPHY

The use of multidimensional GC was originally reported in 1963 [37] and has been under development and use since. Typically, two GC columns which can be packed, open tubular, or combinations of both, are coupled together via switching valves or pneumatic control [38]. Due to the large heat capacity of metal valves, and the possibilities of adsorption or catalytic reactions in the metal parts of a valve [39], valveless switching systems are more commonly used and are based on careful balancing of pressures along the system [38] or flow control [40], and are commonly refered to as Deans switching. Some years later, Schomburg and Weeke [41] developed a live-T-interface which reduces some of the problems encountered in balancing pressures along the multidimensional arrangement.

The use of multidimensional chromatography in the gas phase is perhaps the most widely used multidimensional technique due to the following reasons: mobile phase compatibility, availability of a wide range of sensitive and selective detectors, commercially available instrumentation or add-on accessories and conversion kits to carry out switching operations, and highest total theoretical peak capacity when using columns of capillary dimensions. Some of the disadvantages of multidimensional GC are that components must be sufficiently volatile to be transported in the gas phase (although derivatization techniques alleviate some of these problems [42]); the need for a relatively clean sample so as not to deteriorate the performance of the primary column by contamination with non-volatile or highly polar compounds (particularly when the primary separation is performed using a column of capillary dimensions), the lack of selectivity dependance on mobile phase composition and the limited selectivity differences which are obtained when using common stationary phases. For example, separations of components using stationary phases with very different characteristics, such as methylsilicone and cyanopropyl, are still highly correlated by boiling point. For this reason, selectivity tuning [43] should be considered an important tool for the selection of conditions suitable for multidimensional GC. Ex-

amples of coupled column GC in the petroleum [44,45] geochemical [46], and environmental [47] fields illustrate the utility of multidimensional GC [48,49].

## 5. MULTIDIMENSIONAL LIQUID CHROMATOGRAPHY

In contrast to GC, LC offers extended flexibility, since the mobile phase composition can be adjusted in order to obtain enhanced resolution. The greater selectivity differences between columns which are attainable due to the wide variety of separation modes available, such as for example adsorption, partition, size-exclusion, ion-exchange or affinity chromatography make multidimensional LC a very powerful tool. Highly polar and non-volatile compounds can be separated using most of the above modes, while relatively complex samples can be introduced without severely deteriorating the performance of the system. Some limitations are that the total theoretical peak capacities in multidimensional LC are lower than in multidimensional GC, detection systems are generally not as sensitive or universal as in GC, and mobile phase incompatibilities can limit the applicability of multidimensional LC. Most separation modes can be easily interfaced when the mobile phases used are compatible. The interfacing of normal-phase and reversed-phase systems is particularly difficult, due to the mobile phase immiscibilities. Two approaches have been used to overcome this problem. Sonnefeld *et al.* [50] used a system in which the fraction of interest was transferred from the first (normal-phase) column to a packed precolumn, and the normal-phase eluent was removed by passage of an inert gas and vacuum. Once the solvent was removed, the precolumn was desorbed using a reversed-phase eluent and transfered to the second (reversed-phase) analytical column. More recently Takeuchi *et al.* [51] used a microcolumn in the first dimension and a conventional-size column in the second dimension to interface normal phase and reversed-phase separations. Due to the reduced peak volume generated by the use of microcolumns, solvent removal was not required.

Another example of the flexibility attainable by the use of microcolumns is in the coupling of size-exclusion chromatography (SEC) to reversed-phase chromatography for the determination of polymer additives [52], as illustrated in Figs. 4 and 5. In this case, the use of a conventional-size column in the first dimension would have yielded a fraction containing the additives of interest in a volume of *ca.* 1 ml. Introduction of such a large volume of tetrahydrofuran into an aqueous mobile phase would have yielded broadened and distorted peaks. Because of the lowered volumetric dispersion obtained by the use of microcolumns, the additive fraction obtained was only 6 $\mu$l, a volume which was easily introduced into the reversed-phase system without peak shape deterioration or resolution losses.

Examples of multidimensional LC in the petroleum [53], pharmaceutical [54], biomedical [55] and toxicological [56] areas have been presented, and a review of the technique was recently published [57].

## 6. MULTIDIMENSIONAL LIQUID CHROMATOGRAPHY–CAPILLARY ELECTROPHORESIS

The coupling of LC and capillary electrophoresis (CE) was recently described by Bushey and Jorgenson [58,59]. As CE operates under fundamentally different separation mechanisms, the combination with LC represents a true orthogonal system. A reversed-phase LC system was used in the first dimension, and eluting fractions were introduced and further separated on a CE system, which was used to separate peptide standards and fluorescently labeled peptide fragments from a tryptic digest of ovalbumin [58] and to compare tryptic digest fingerprints of horse heart cytochrome *c* and bovine heart cytochrome *c* [59]. A diagram of the experimental

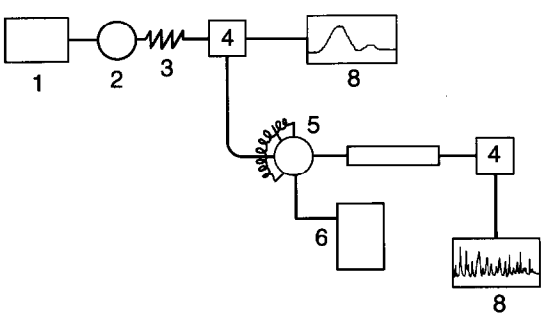


Fig. 4. Schematic diagram of micro SEC–LC system. 1 = Micro LC pump; 2 = injection valve; 3 = micro SEC column; 4 = detector; 5 = switching valve; 6 = LC pump; 7 = LC column; 8 = recording devices [52].

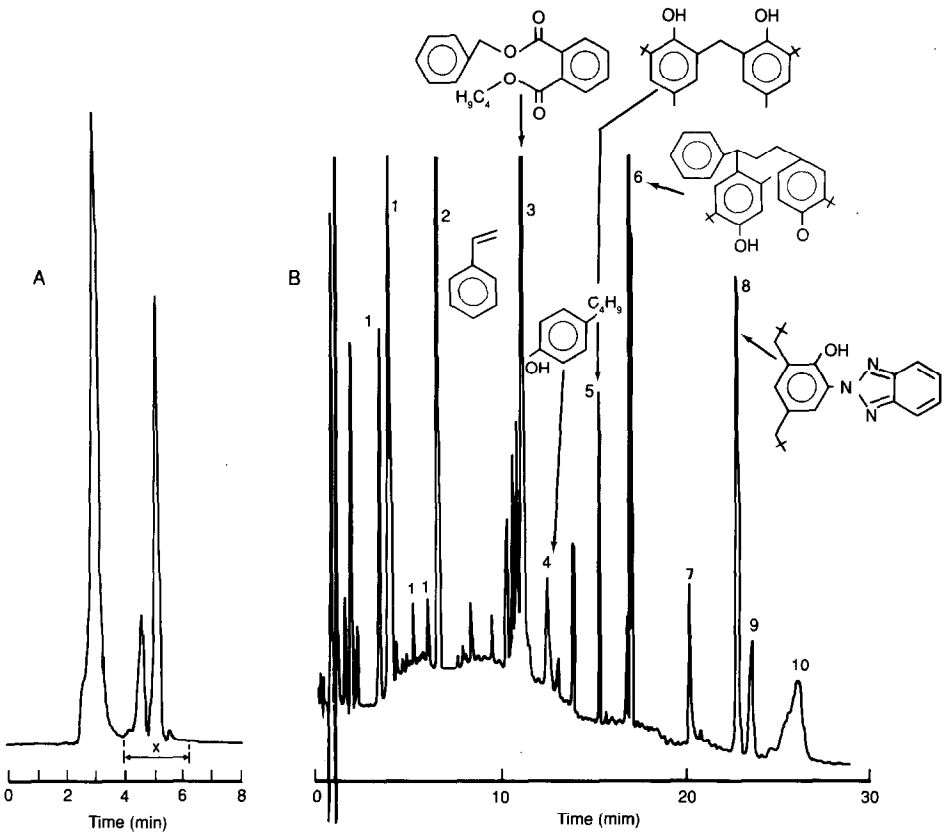


Fig. 5. Micro SEC–LC chromatograms of acrylonitrile–butadiene–styrene sample. (A) Micro SEC. Column: 30 cm × 250 mm I.D. fused silica packed with PL-GEL, 50 Å pore-size, 5 mm particle diameter. Eluent: tetrahydrofuran. Flow: 2.0 ml/min. Injection size: 200 nl. Detection: UV at 254 nm. x = polymer additive fraction transfered to LC system (*ca.* 6 μl). (B) LC chromatogram of introduced fraction. Column: 15 cm × 4.6 mm I.D. Nova-Pak $C_{18}$. Eluent: acetonitrile–water (60:40) to (95:5) in 15 min gradient. Flow: 1.5 ml/min. Detection: UV at 214 nm. Peaks: 1 = styrene–acrylonitrile oligomers; 2 = styrene; 3 = benzylbutyl phthalate; 4 = nonylphenol isomers; 5 = Vanox 2246; 6 = Topanol CA; 7 = unknown; 8 = Tinuvin 328; 9 = Irganox 1076; 10 = unknown [52].

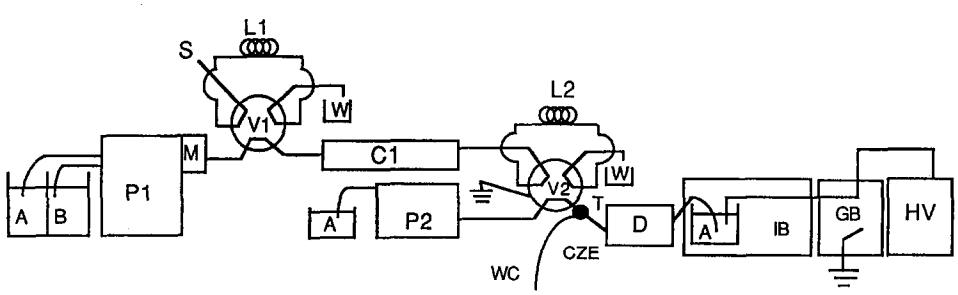


Fig. 6. Schematic of two-dimensional high-performance LC–CE instrumentation. A and B = buffer A and acetonitrile respectively; P1 = Brownlee microgradient syringe pump; M = 52-μl mixer; V1 = Valco six-port manual injection valve; S = injection syringe; L1 = 50-μl loop; C1 = reversed-phase column; P2 = Waters Assoc. Model 6000A piston pump; V2 = grounded six-port electrically actuated Valco valve; L2 = 10-μl loop; CZE = CE capillary; T = Valco low-dead-volume tee; WC = waste capillary; D = fluorescence detector; IB = interlock box; GB = grounding box; HV = Spellman high-voltage power supply; W = waste [59].

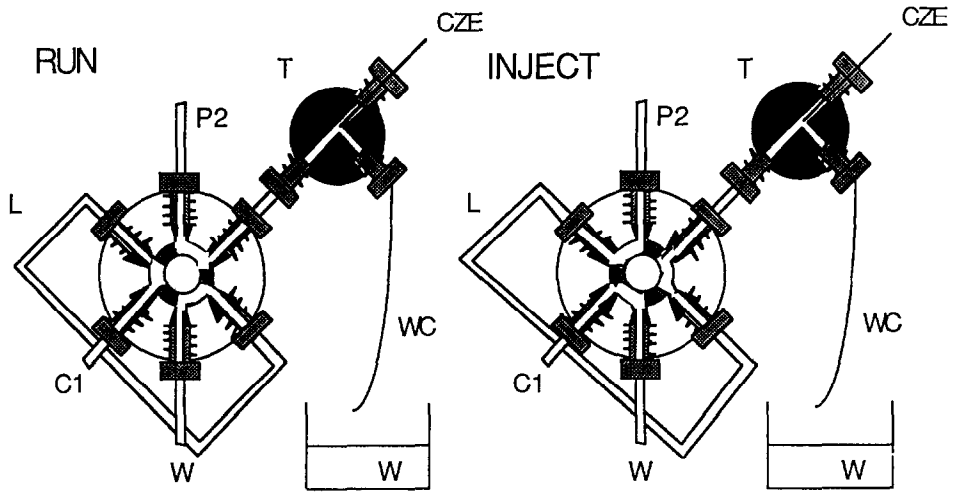


Fig. 7. LC–CE coupling: valve connections. C1 = Reversed-phase (RP) high-performance LC column; P2 = pump 2; L = loop; CZE = capillary electrophoresis fused-silica capillary; WC = waste capillary; T = Valco low-dead volume tee; W = waste [59].

setup is presented in Fig. 6, while a detail of the valving configuration is presented in Fig. 7. In the operation of the system, the LC effluent fills a loop, and the contents are passed through a tee where the end of the CE capillary is positioned. Sample is introduced into the CE system by electromigration. By using reduced diameter capillaries, high voltage drops per unit length could be applied, yielding increased efficiency and shorter analysis times. A three-dimensional representation of the separations obtained is presented in Fig. 8.

## 7. MULTIDIMENSIONAL SUPERCRITICAL FLUID CHROMATOGRAPHY

Supercritical fluids have physical properties between those of liquids and gases, and as mobile phases for chromatography, solvent strength is closely dependent on density [60]. Therefore, variations in density allow chromatographic behaviour which becomes more "GC-like" or "LC-like" depending on the pressure and temperature conditions chosen. The coupling of SFC to capillary GC (or another SFC) offers an advantage in that supercritical fluids are generally more compatible than liquids, as they typically decompress into gases under GC conditions. However, to a large extent, selectivity is controlled by the stationary phases used. The increased use of polar modifiers [61] and the development of stationary phases with unique characteristics, such as liquid crystalline phases [62] and chiral stationary phases [63], suggest great potential for resolution of complex samples using SFC in a multidimensional mode. Various examples of the application of multidimensional SFC to the separation of a complex matrices have been presented [64–

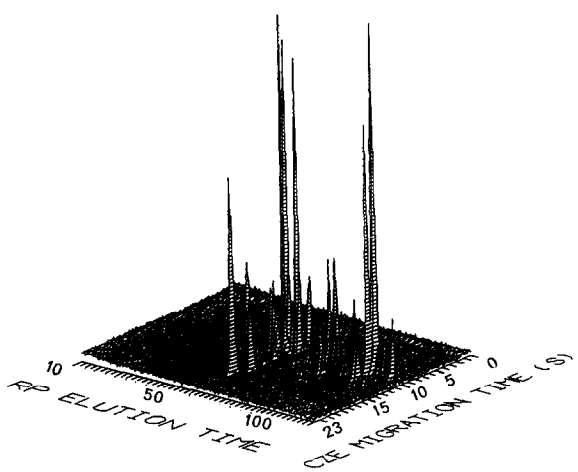


Fig. 8. Three-dimensional plot of horse heart cytochrome *c*. Obtained with 15 μm I.D. capillary. CE injections: −1 kV, 5 s; CE runs: −28 kV, 0.5 min; P1 flow-rate: 20 μl/min; P2 flow-rate: 0.3 ml/min; CE capillary 6.5 cm to detector, 26 cm overall. Eight points per second collected. Every other point displayed for injections 20–245 [59].

66]. Of particular interest, the analysis of a bird gullet extract utilizing columns of capillary dimensions was published [67] as well as the analysis of a complex hydrocarbon matrix using packed microcolumns [68].

## 8. MULTIDIMENSIONAL LIQUID CHROMATOGRAPHY–CAPILLARY GAS CHROMATOGRAPHY

The coupling of a liquid chromatograph to a gas chromatograph in an on-line mode offers another, different perspective on multidimensional separations. As with LC–CE, an orthogonal operation system is realized. Selectivities that are difficult to obtain using gas or liquid phases alone are in principle possible using the wide range of variables available, such as mobile and stationary phases, temperature profiles and detector systems of the two techniques. A system of this type combines the selectivity of LC with the efficiency and sensitivity of GC, yielding high peak capacities. In the application of this technology, the LC can act as an efficient clean up step, yielding a much less complex fraction for subsequent GC analysis, or as a chemical class fractionation step, where group types can be transferred to the GC for individual separation of the components within each class. LC–GC also provides two independent retention data sets, which can be helpful in confirming the identity of unknown components.

Matching LC and GC presents several challenges, since the two separation techniques operate in phases which are in two different physical states, and the relatively large volume of LC effluent must be made compatible with the gas chromatograph. As discussed above, the peak elution volumes of a liquid chromatograph can range from a few microliters when using LC columns of less than 1 mm diameter (microcolumns) up to several milliliters when using conventional size columns. In either case, the volumes introduced are larger than can normally be tolerated in capillary GC using on-column injection techniques. The successful interfacing of LC and GC involves the steps of isolation of the fraction containing the components of interest, transfer of the isolated fraction to the gas chromatograph and volatilization of the solvent and of the components of interest.

The basic approaches which have been used to introduce effluent from a liquid chromatograph to a gas chromatograph are to introduce a sufficiently small volume of the peak of interest from the LC so that the injected profiles of the components of interest are not distorted by the large volumes of solvent [69], to develop introduction techniques which allow large volumes of effluent to be introduced into the GC [70,71] and to reduce the LC column diameter in order to elute the components of interest in a smaller volume [71].

Effluent from the LC system can be directed to the GC system by interposing a switching valve (four- or six-port) between the LC and the GC uncoated inlet/capillary column. The components of interest are bypassed to waste when the valve is in one position, and transferred to the GC injector (or directly to the uncoated inlet) when the valve is switched to the alternate position. After the transfer is complete, the connecting tube between the valve and GC injector may be backflushed to decrease the probability of contamination of the next section transfered from the LC column. If the system does not involve the GC injector, the transfer line is flushed by the eluting mobile phase and backflushing is not required. The nomenclature of "stop-flow introduction" is suggested for the process of interrupting carrier gas flow to the uncoated inlet/capillary GC column arrangement while the LC effluent is introduced. The time period for which carrier gas flow is interrupted can be relatively long, as when the effluent is introduced by the LC pump, or relatively short, as when the effluent is trapped in an external valve loop and introduced via carrier gas flow pushing the contents of the loop into the GC system. In either case the main variables that affect the quality of the results obtained are the introduction temperature of the effluent and the introduction rate. The introduction of solvent into the GC system under conditions in which carrier gas is introduced at the same time as the effluent from the LC system should be considered to be different, and for clarity purposes we suggest the term "simultaneous introduction".

As mentioned above, stop-flow introduction can also be accomplished using a loop injection. A six- or ten-port valve is connected to the LC detector outlet, and the components are introduced into a fixed loop of known volume, corresponding to the volume of the fraction of interest. When the valve is

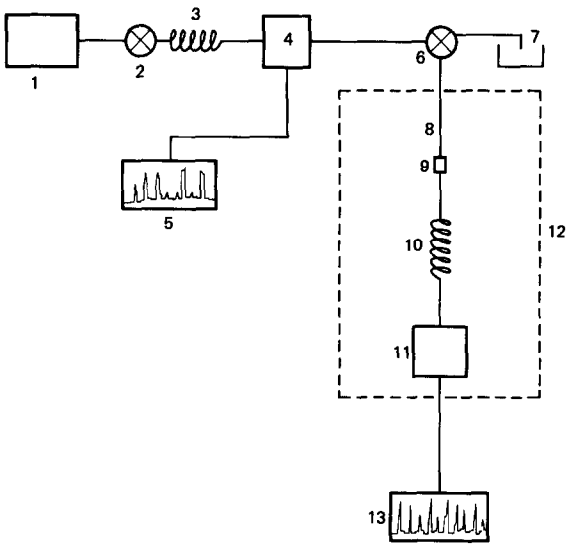


Fig. 9. Schematic diagram of LC–GC system. 1 = pump; 2 = injector; 3 = LC column; 4 = UV detector; 5 = recorder; 6 = switching valve; 7 = waste; 8 = uncoated inlet; 9 = butt connector; 10 = capillary GC column; 11 = detector; 12 = GC oven; 13 = recorder [71].

switched, the carrier gas flushes the sample loop and forces the liquid plug into the GC column. If a ten-port switching valve is used, a second loop can be added to either introduce other components into the GC column or to flush the sample loop if the second loop is filled with solvent in order to decrease contamination.

A schematic diagram of an LC–GC system is presented in Fig. 9, while valving configurations used to couple the two separation systems are presented in Fig. 10.

A number of applications of LC–GC have been published, where the primary separation step is conducted using LC columns of conventional dimensions [72–87]. The ease of use of conventional-size columns for LC demands however an increase in complexity of the interface design [88,89] due to the effluent volumes involved, and precludes the use of reversed-phase (aqueous) systems [90]. Since a large majority of the LC separations performed today are done in the reversed-phase mode, this is an important limitation to the use of conventional size columns in the first dimension. In contrast, the use of microcolumns simplifies the liquid introduction process, allowing introduction of aqueous eluents without the severe difficulties encountered using conventional size columns [91–105]. Examples of such applications are the determination of an insecticide in a supercritical fluid extract of wheat, as shown in Fig. 11, and the determination of a herbicide in soil, presented in Fig. 12.

### *8.1. Quantitative determination of polymer additives*

An example of the power of multidimensional

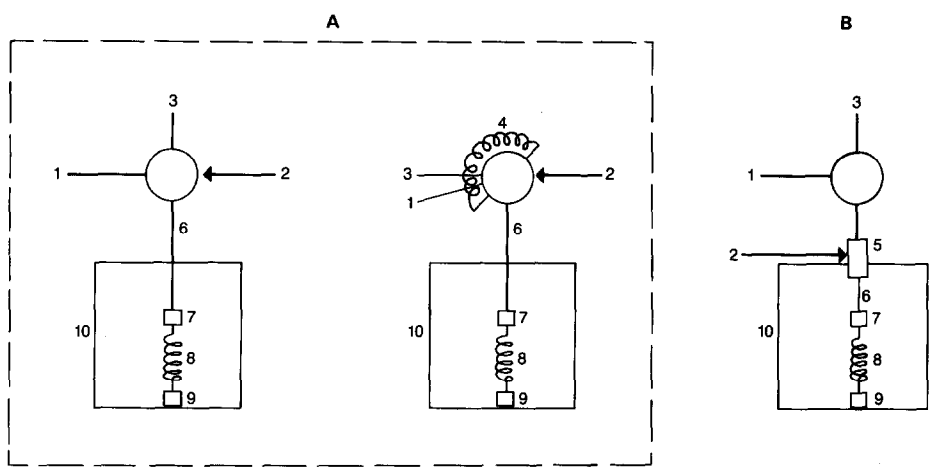


Fig. 10. Representation of LC–GC interfaces. (A) Stopped-flow introduction. (B) Simultaneous introduction. 1 = LC effluent; 2 = carrier gas; 3 = LC waste; 4 = external sample loop (volume equivalent to transfered section); 5 = GC injector; 6 = uncoated inlet; 7 = dead volume free connector; 8 = capillary GC column; 9 = detector; 10 = GC oven [102].

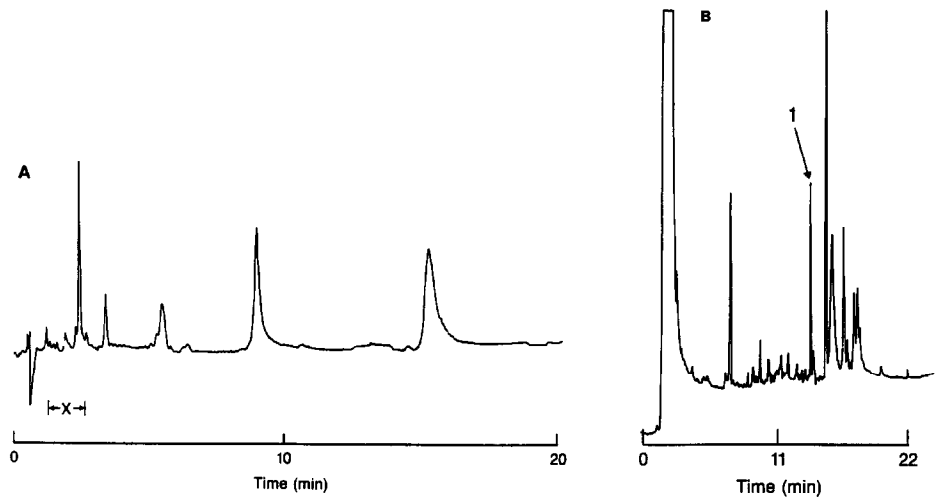


Fig. 11. LC–GC separations of supercritical fluid extract of wheat. (A) Micro LC. Column: 40 cm × 250 μm I.D. fused silica packed with Spherisorb ODS, 5 μm particle diameter; eluent: acetonitrile–water (85:15); flow: 6 μl/min; detection: UV at 214 nm; injection: 60 nl; X = fraction introduced into capillary GC. (B) Capillary GC of introduced fraction. Column: 30 m × 0.25 mm I.D. DB-5, 0.25 μm film thickness; oven uncoated inlet: 5 m × 0.25 mm I.D. undeactivated fused silica; temperature program: 115 to 270°C at 8°C/min; detection: electron capture; carrier: helium at 28 cm/s; peak 1 = chlorpyrifos methyl (50 ng/g) [95].

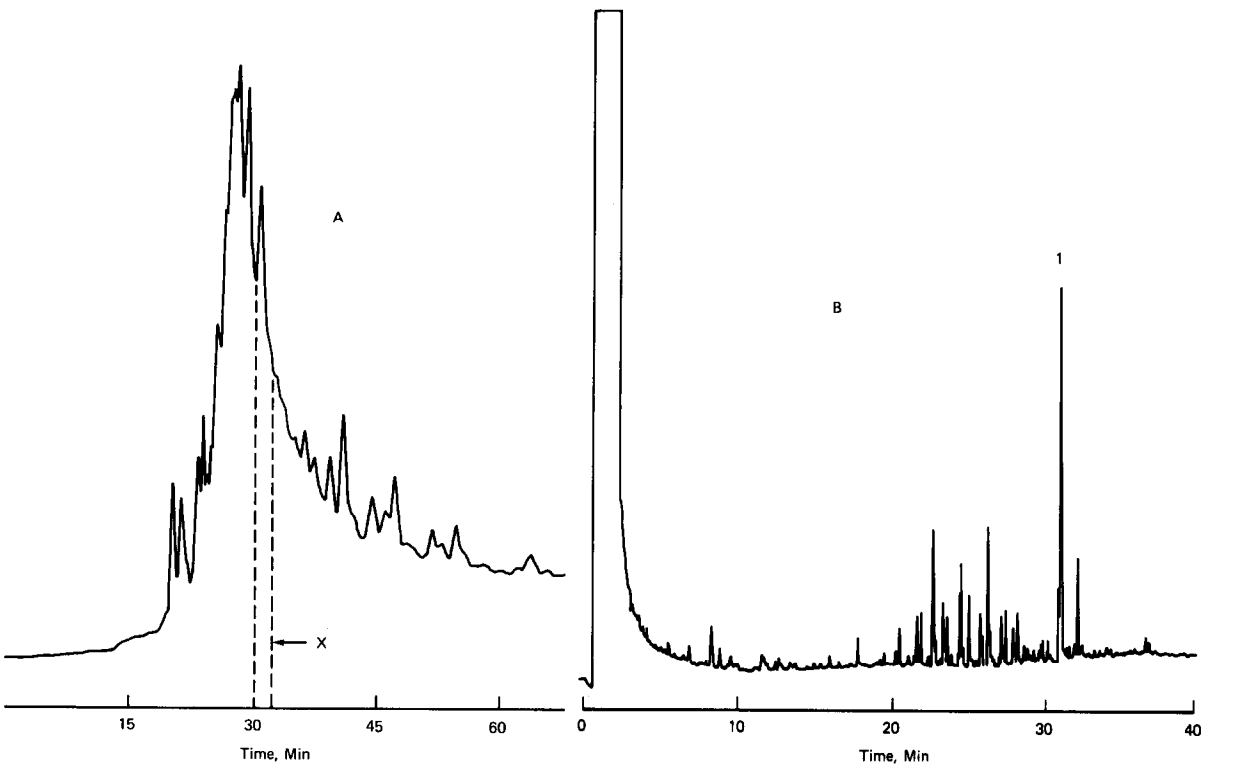


Fig. 12. Chromatograms of soil extract. (A) Micro LC. Column: 105 cm × 250 μm I.D. fused silica packed with Spherisorb ODS, 5-μm particle diameter; mobile phase: methanol–water (90:10); flow: 3.0 μl/min; detection: UV at 214 nm; injection: 200 nl; X = section introduced into the GC. (B) Capillary GC of introduced fraction. Column: 30 m × 0.25 mm I.D. J&W Carbowax, 0.25-μm film; uncoated inlet: 10 m × 0.25 mm I.D.; undeactivated fused silica; oven: 100°C 10 min, 5°C/min to 230°C; carrier: helium at 80 cm/s; detection: flame ionization; peak 1 = 2-chloro-N-isopropylacetanilide (14 μg/g) [92].

separations is in the field of polymer characterization. A variety of additives are typically incorporated into the polymer systems to enhance their enduse performance. Determination of the identity and levels of such additives is typically performed by isolation of the additives via soxhlet extraction, or by dissolving the polymer in a suitable solvent, followed by precipitation of the polymer and analysis of the supernatant using chromatographic techniques and identification via mass spectrometry. However, such sample preparation schemes may not yield accurate quantitative results, due to the solubility dependance of the additives and the probability of coprecipitation with the polymer.

An alternative analysis scheme is to separate the additive fraction from the polymer via microcolumn SEC, followed by on-line introduction into capillary GC with mass spectrometric detection [104]. The techniques developed were applied to a wide variety of commercial polymer products. The main advantage of such a system is that it eliminates the inherent losses when additives are separated from the polymers via other conventional techniques, in addition to minimizing sample analysis times. Typical chromatograms obtained using the technology described are presented in Fig. 13, while Table 1 represents a quantitative comparison of additive concentrations obtained using the precipitation approach and the multidimensional approach. As can be observed, additive losses were experienced using the precipitation approach [105].

### *8.2. Characterization of non-volatile compounds*

In order to overcome one of the limitations of on-line coupled LC–GC, which is the requirement that components be sufficiently volatile to be transported in the carrier gas, an interface was designed which would allow the conversion of non-volatile species to volatile fragments. Alternative approaches are the use of off-line treatments such as derivatization [42], or on-line treatments, such as subjecting the analyte to postcolumn reactions [106].

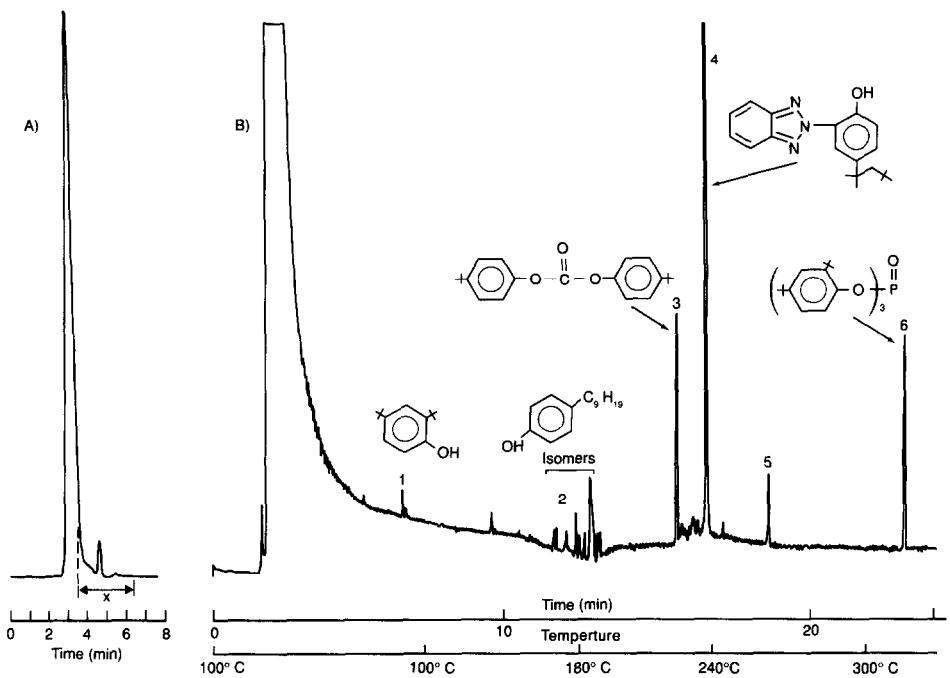


Fig. 13. Chromatograms of polycarbonate sample. (A) Micro SEC. (B) Capillary GC of introduced fraction. Micro SEC conditions as in Fig. 5. Column: 15 m × 0.25 mm I.D. DB-1, 0.25 μm film; uncoated inlet: 5 m × 0.32 mm I.D. deactivated fused silica; Temperature program: 100°C 8 min, 12°C/min to 350°C; detection: flame ionization; X = fraction introduced into capillary GC. Peaks: 1 = 2,4-di-*tert.*-butylphenol; 2 = nonylphenol isomers; 3 = di(4-*tert.*-butylphenyl) carbonate; 4 = Tinuvin 329; 5 = solvent impurity; 6 = Irgaphos 168 (oxidized) [104].

TABLE 1

POLYCARBONATE ADDITIVE ANALYSIS

R.S.D. = Relative standard deviation.

| | Concentration (μg/g) | |
|---|---|---|
| | Precipitation (R.S.D. %) | Micro SEC–GC (R.S.D. %) |
| 2,4-Di-*tert.*-butylphenol | 60 (15) | 80 (3.8) |
| Di-(4-*tert.*-butylphenyl) carbonate | 620 (9.0) | 630 (4.5) |
| Irgaphos 168 | 570 (9.5) | 540 (6.3) |
| Tinuvin 329 | 2680 (7.9) | 3530 (5.1) |

The development of a pyrolysis interface to effect a postcolumn treatment of a non-volatile material (in this case a styrene–acrylonitrile copolymer) was published [107]. Fig. 14 represents a diagram of the multidimensional system while Fig. 15 represents details of the interface design.

Polymer characterization, in terms of composition *vs.* molecular mass is valuable information which aids in the understanding of polymerization chemistry. Some of the approaches used to obtain this type of information have included the use of adsorption chromatography [108,109], gradient elution LC [110,111], precipitation chromatography [112] or adsorption chromatography followed by size exclusion [113]. The analysis of compositional and structural heterogeneities of polymers by non-exclusion LC has also been reviewed [114].

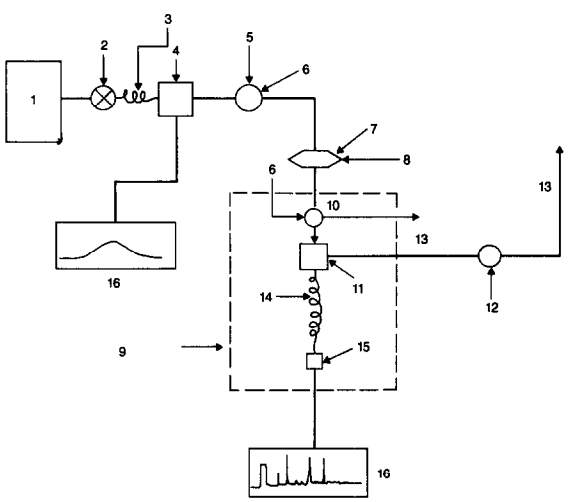


Fig. 14. Schematic diagram of on-line LC–pyrolysis–GC system. 1 = Pump; 2 = injection valve; 3 = micro LC column; 4 = detector; 5 = ten-port switching valve; 6 = carrier gas; 7 = interface; 8 = auxiliary carrier; 9 = GC oven; 10 = four-port switching valve; 11 = splitter; 12 = micro metering valve; 13 = vent; 14 = GC column; 15 = detector; 16 = recorder [107].

The characterization of a styrene–acrylonitrile copolymer was accomplished by separating the polymer via microcolumn SEC, transferring selected fractions of the molecular weight distribution to an interface, and subjecting the polymer in the sections selected to pyrolysis–GC, in order to determine the relative composition of the isolated fractions by the ratio of the monomeric composition obtained upon pyrolysis. A study of the variables influencing reproducibility, such as interface temperature, flow, and pyrolysis ribbon geometry were conducted [107]. Typical chromatograms obtained are presented in Fig. 16.

## 9. SUPERCRITICAL FLUID EXTRACTIONS

The application of multidimensional chromatography to the analysis of complex matrices helps to minimize sample pretreatment steps. Still, when the matrix to be analyzed is not totally soluble in a particular solvent, such as for example plant tissue, a preliminary step is necessary to obtain a solution suitable for subsequent introduction into the preliminary separation stage. Supercritical fluids offer potential advantages over liquid solvents to meet the sample preparation requirements. The solvent strength of supercritical fluids approach those of liquid solvents while having lower viscosities and higher solute diffusivities. Further, the solvent strength of a supercritical fluid increases with in-

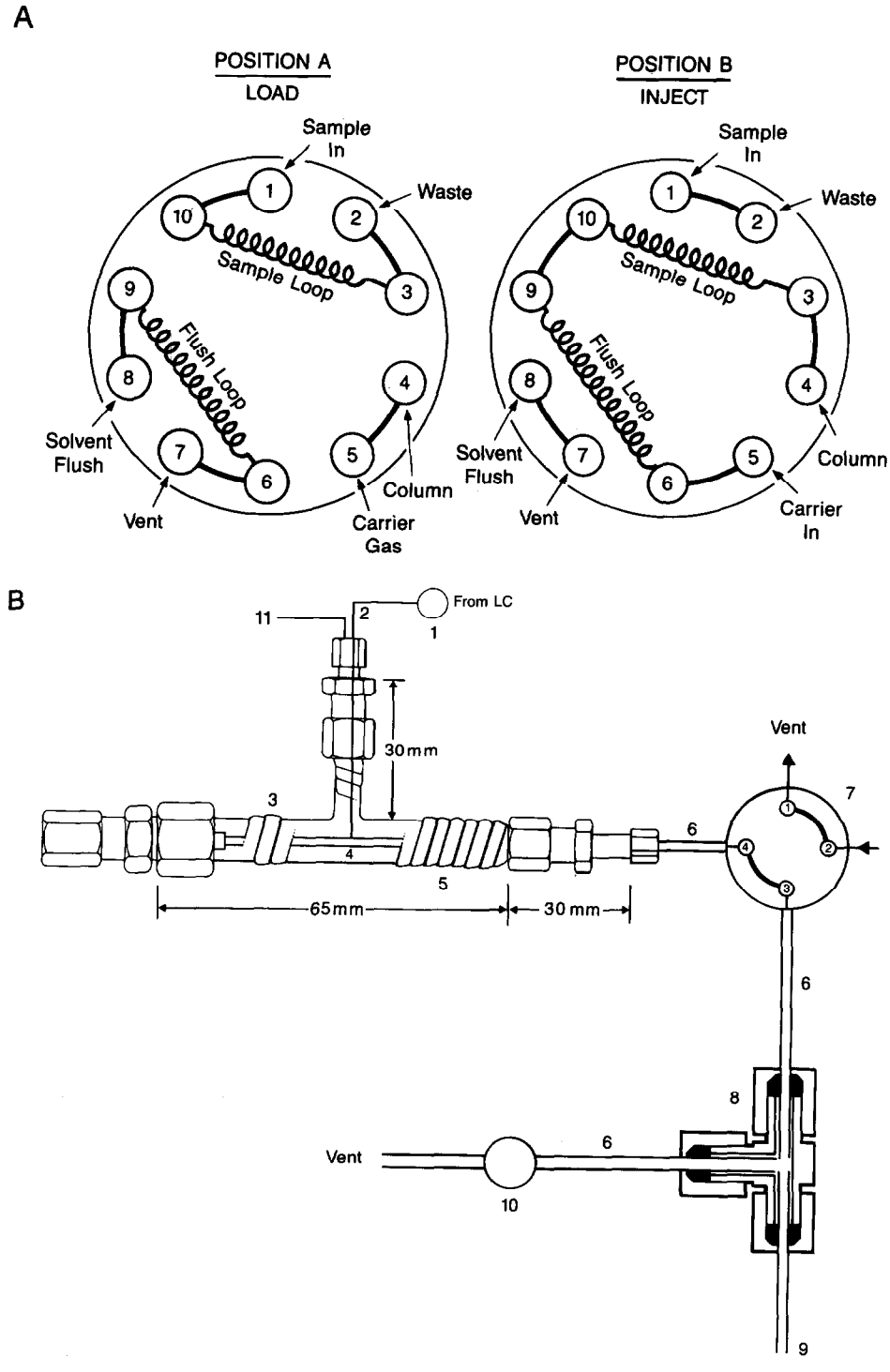


Fig. 15. Diagram of LC–pyrolysis–GC interface. (A) Ten-port switching valve and loop configuration. (B) Glass chamber interface: 1 = ten-port switching valve; 2 = transfer capillary; 3 = glass chamber; 4 = pyrolysis ribbon; 5 = heating tape; 6 = transfer capillaries; 7 = four-port switching valve; 8 = split tee; 9 = capillary GC column; 10 = micrometering valve; 11 = auxiliary carrier gas [107].

creasing density [115,116]. The conditions necessary to extract target analytes can therefore be optimized by varying the extraction pressure, the extraction temperature or both. In addition, many supercritical fluids are gases at standard temperatures and pressures, which simplifies the concentration and collection of extracted analytes.

Supercritical fluids have been used for extraction purposes on an industrial-scale process for several years [117,118] but as a sample preparation technique for chromatography its use is a relatively recent development [119]. Supercritical fluid extraction (SFE) was used in conjunction with thin-layer chromatography [120] and conventional LC [121]. The on-line coupling of SFE to capillary GC [122–126] and capillary SFC [127–132] is experiencing rapid growth and will continue to be studied as a simplified method of sample preparation and analysis. To our knowledge, however, the reported studies on analytical SFE have typically dealt with analyte concentrations in the μg/g range, orders of magnitude higher than necessary to study pesticide residues, for example, which are determined at the ng/g level. Studies were conducted in the SFE of a pesticide from a wheat matrix at these concentrations [95]. It was discovered that the extracts generated for analysis at the ng/g level from this matrix were not sufficiently clean (interference-free) to be analyzed directly by capillary GC alone, and microcolumn LC–GC was required in order to analyze the extracts obtained [95].

When performing SFE off-line, extracted components are typically collected in a solvent, a portion of which is then introduced into a chromatographic system. This approach does not take full advantage of the potential sensitivity increases which can be obtained in an on-line system. For example, if 100% of the analyte of interest can be extracted from the matrix, and no losses of analyte occur during the transfer process, the total mass of analyte extracted will reach the detector, yielding optimal sensitivity. In addition, a system of this type would allow analyses on very small sample sizes.

In order to decrease sample handling steps and to increase the sensitivity of the analyses, a system was developed coupling SFE on-line to microcolumn LC–GC [133]. A schematic diagram of the system developed is presented in Fig. 17, and the impactor

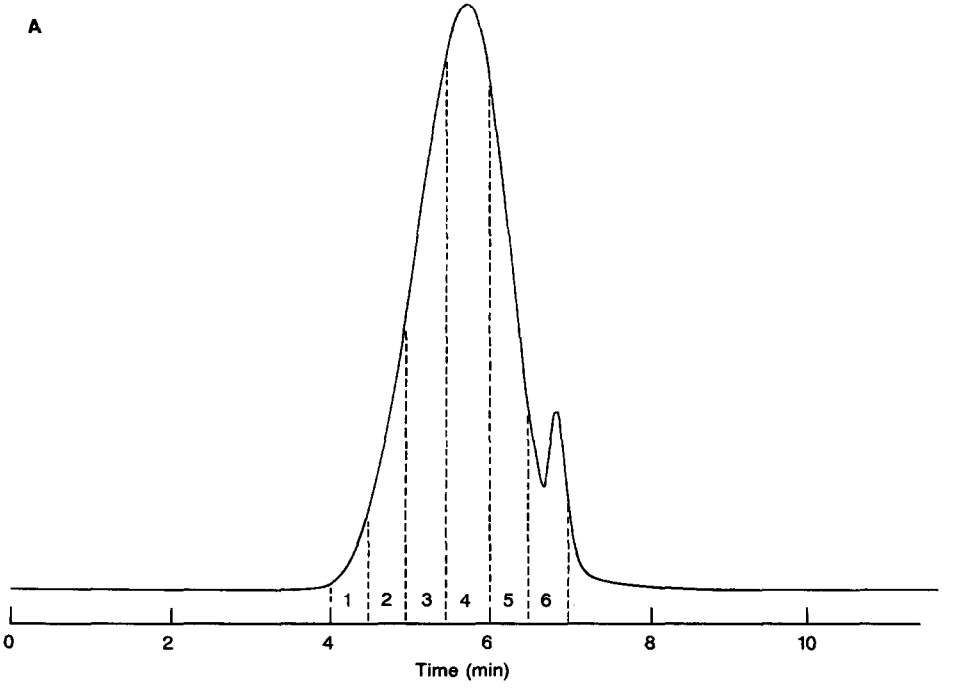


Fig. 16.

(Continued on p. 18)

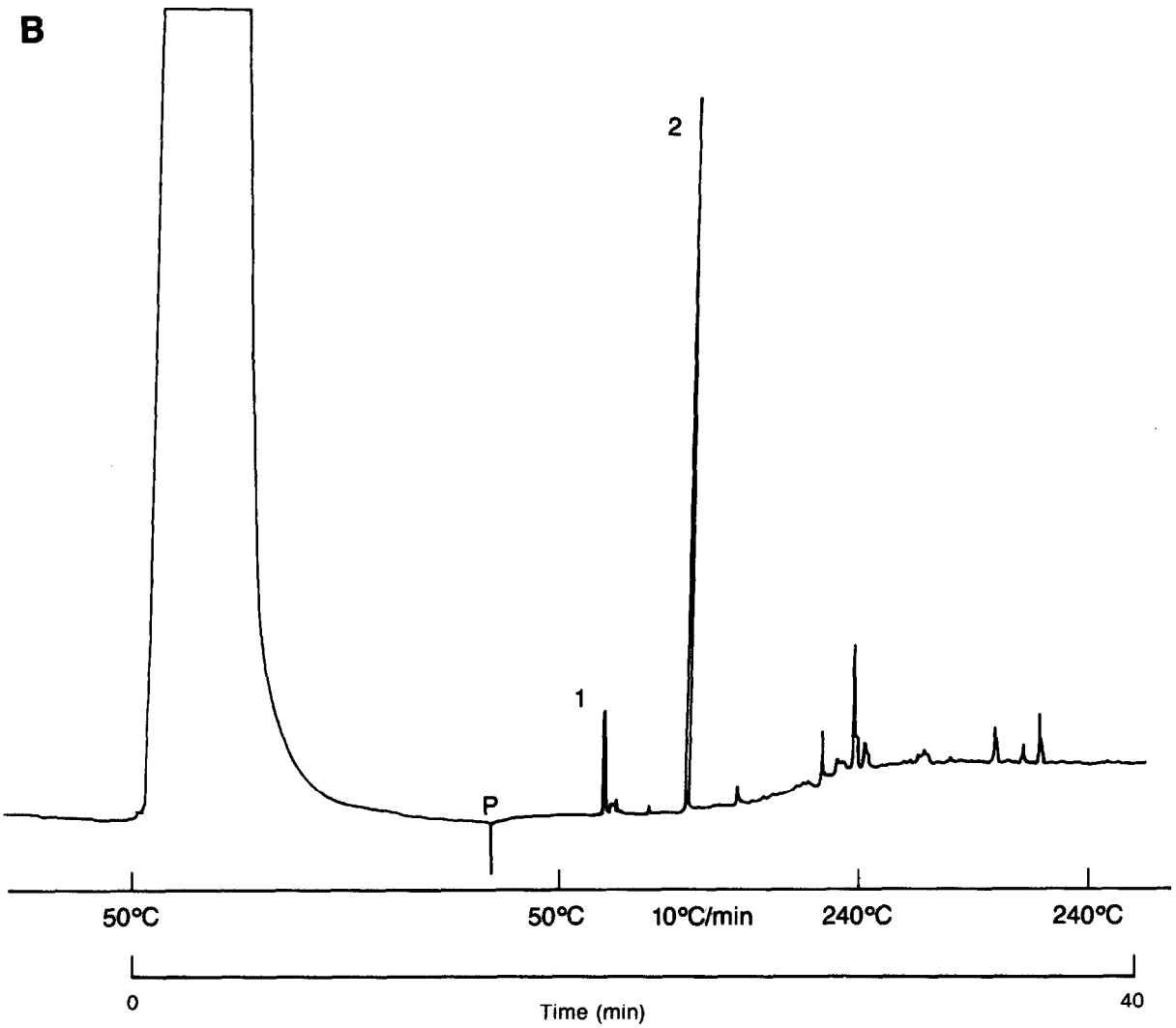


Fig. 16. (A) Micro SEC chromatogram of styrene–acrylonitrile copolymer. Column: 50 cm × 250 μm I.D. fused silica packed with Zorbax PSM-1000, 7-μm particle size; eluent: tetrahydrofuran; flow: 2.0 μl/min; injection: 200 nl; detection: UV at 220 nm. Fractions transferred to the pyrolysis interface are indicated. (B) Pyrolysis–GC chromatogram of introduced fraction from micro SEC. Column: 30 m × 0.2 mm I.D. phenylmethyl silicone, 0.33 μm film; temperature program: 50 to 240°C at 10°C/min; carrier: helium at 60 cm/s; detection: flame ionization. Peaks: 1 = acrylonitrile; 2 = styrene; P = pyrolysis time [107].

interface used is presented in Fig. 18. In the operation of the system, a sample is placed in a vessel and extracted with supercritical carbon dioxide. The extracted components are deposited in the impactor interface as a narrow band by decompressing the fluid into the gas phase via a restrictor and providing a surface for further dissipation of kinetic energy. The deposited material is then transferred to the micro LC column, where the target analyte(s) are separated from the majority of co-extracted interferences. At the appropriate time, the LC fraction containing the components of interest is introduced into the capillary GC system, where further separation and detection takes place. The system was applied to the determination of an insecticide in grass samples at the ng/g level. Reproducibility ex-

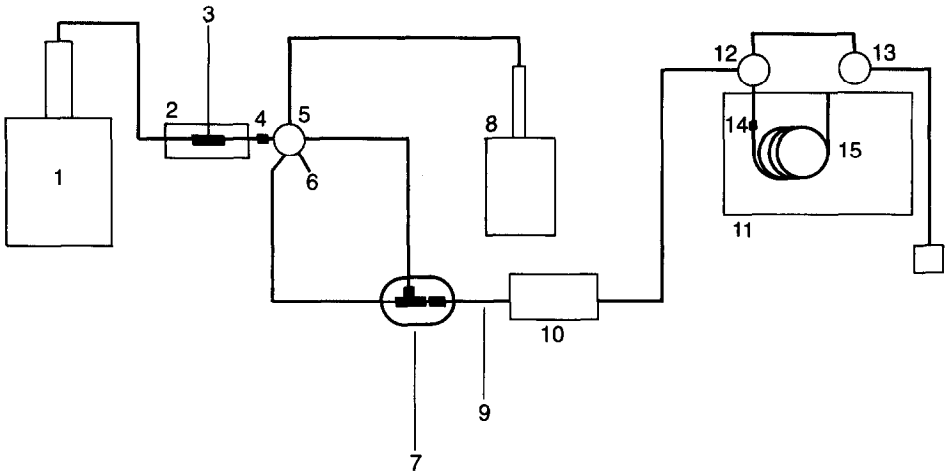


Fig. 17. Schematic diagram of on-line supercritical fluid extraction–microcolumn LC–capillary GC system. 1 = Supercritical fluid pump; 2 = column heater; 3 = extraction vessel; 4 = filter; 5 = switching valve (V1); 6 = vent; 7 = impactor interface; 8 = micro LC pump; 9 = micro LC column; 10 = UV detector; 11 = GC oven; 12 = switching valve (V2); 13 = on–off valve (V3); 14 = uncoated inlet; 15 = capillary GC column [133].

periments yielded R.S.D. values of 10.8% with sample sizes of only 5 mg and total organic solvent usage of less that 100 $\mu$l. Representative chromatograms are presented in Fig. 19.

## 10. MULTIDIMENSIONAL LIQUID CHROMATOGRAPHY–CAPILLARY SUPERCRITICAL FLUID CHROMATOGRAPHY

The coupling of LC to capillary SFC is expected to be of utility in the characterization of complex samples where components of interest are thermally labile, do not contain significant chromophores or do not have sufficient volatility to be analysed by GC. Since capillary columns of 50 $\mu$m I.D. are necessary for optimal chromatographic performance, injection volumes are typically in the nanoliter range, yielding limited sensitivity. Alternative sample introduction processes which would allow larger sample volumes into capillary SFC columns have been investigated, such as the use of a dilution chamber [134], solvent venting techniques [135–137], density gradient focusing [138] or solvent backflushing [138].

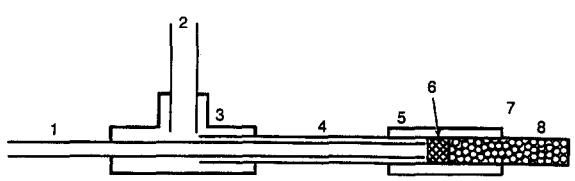


Fig. 18. Schematic diagram of impactor interface. 1 = Linear restrictor; 2 = LC eluent inlet/$CO_2$ vent; 3 = low-dead-volume tee; 4 = impactor tube; 5 = low-dead-volume union; 6 = impactor; 7 = micro LC column; 8 = packing [133].

Multidimensional LC–SFC has been reported using conventional-size columns in the first dimension, so that a small fraction of the peak of interest was transferred to the SFC, allowing for qualitative results only [139]. More recently, LC–SFC was reported using the dilution chamber approach [140] and combinations of the above techniques [141].

An alternative approach was recently developed [142] which allowed introduction of hundreds of microliters of solvent into capillary SFC columns without detrimental effects on peak shapes and resolution. A schematic diagram of the system is presented in Fig. 20. In the operation of the system, the liquid fraction containing the components of interest eluting from the LC system is introduced into an uncoated inlet, where the solvent is removed by heat and passage of an inert gas. Once the solvent is eliminated, the components of interest which are deposited in the inlet are transferred to the interface by extraction of the inlet with supercritical carbon dioxide. The supercritical carbon dioxide stream is

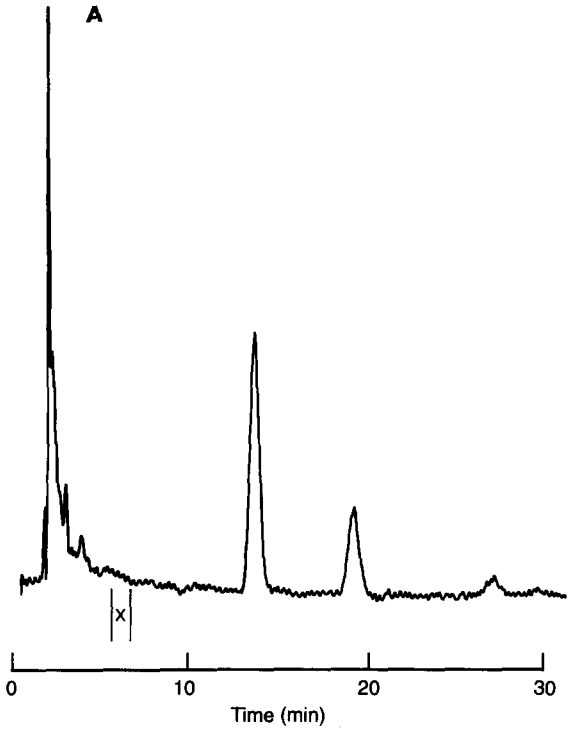


Fig. 19. (A) Micro LC chromatogram of supercritical fluid extract of grass. Column: 20 cm × 250 μm I.D. fused silica packed with Spherisorb ODS; eluent: methanol–water (80:20); flow: 4.1 μl/min; detection: UV at 205 nm; injection: 60 nl; X = fraction introduced into capillary GC. (B) Capillary GC of fraction introduced from Micro LC. Column: 30 m × 0.25 mm I.D. DB-1, 0.25 μm film; program temperature: 115°C 6 min, 8°C/min to 300°C; detection: electron-capture. Peak 1 = chlorpyrifos (160 ng/g) [133].

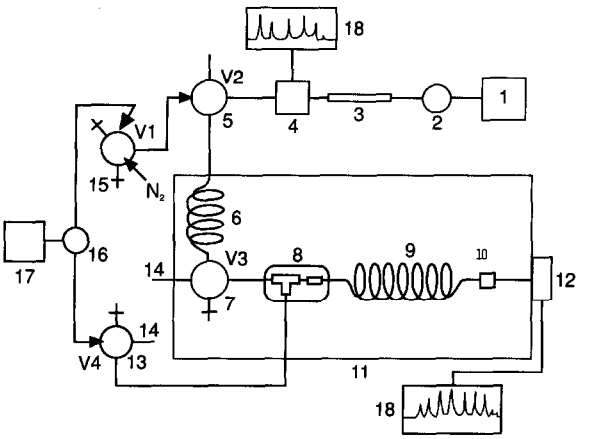


Fig. 20. Diagram of LC–capillary SFC system. 1 = Pump; 2 = injection valve; 3 = micro LC column; 4 = detector; 5 = ten-port switching valve (V2); 6 = capillary inlet; 7 = four-port switching valve (V3); 8 = impactor interface; 9 = capillary SFC column; 10 = frit restrictor; 11 = GC oven; 12 = flame ionization detector; 13 = four-port switching valve (V4); 14 = vents; 15 = ten-port switching valve (V1); 16 = tee; 17 = SFC pump; 18 = recording devices [142].

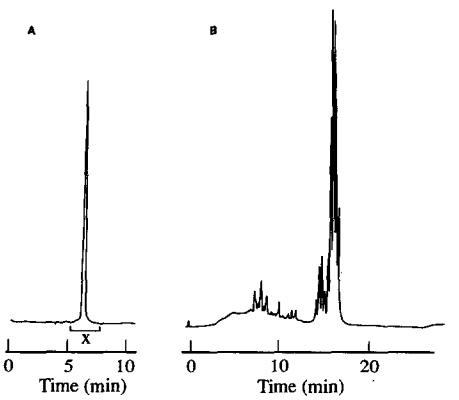


Fig. 21. Chromatograms of pentaerythritol tetrastearate. (A) Micro SEC. Column: 30 cm × 250 μm I.D. PL-GEL, Ångstrom 5 μm particle size; flow: 2.0 μl/min; eluent: tetrahydrofuran; detection: UV at 254 nm; injection size: 60 nl; X = section introduced into capillary SFC. (B) SFC chromatogram of introduced section. Volume introduced: 9 μl; transfer mode; oven temperature 100°C, Program: 100 to 400 atm at 50 atm/min, 5 min final time; Elution mode: column: 10 m × 50 μm I.D. SB-Methyl-100, 0.25 μm film; oven temperature: 100°C, Program: 100 to 400 atm at 15 atm/min, 5 min final time; detection: flame ionization [142].

decompressed though a restrictor, depositing the analytes at the head of the analytical column. When the extraction is completed, the deposited analytes are chromatographed on the SFC system. Chromatograms obtained for pentaerythretol tetrastearate, a polymer additive which is not sufficiently volatile to be analyzed by GC are presented in Fig. 21.

## 11. CONCLUSIONS

The use of multidimensional separation systems has experienced significant growth in the past decade, as is expected to continue to be a fruitful area of research in the future. A large range of options has only just begun to be considered [2]. The theoretical and practical advantages of multidimensional separation systems are beginning to be fully exploited, and it is expected that growth will continue as more users recognize that multidimensional separations can be the most succesful approach to the solution of difficult separation problems.

The use of microcolumns in multidimensional separations has allowed coupling of seemingly incompatible techniques, as well as operation of systems close to the theoretical limits. The key role played by miniaturization is expected to be fully exploited in the future.

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