Advancing the Science of Column Selectivity

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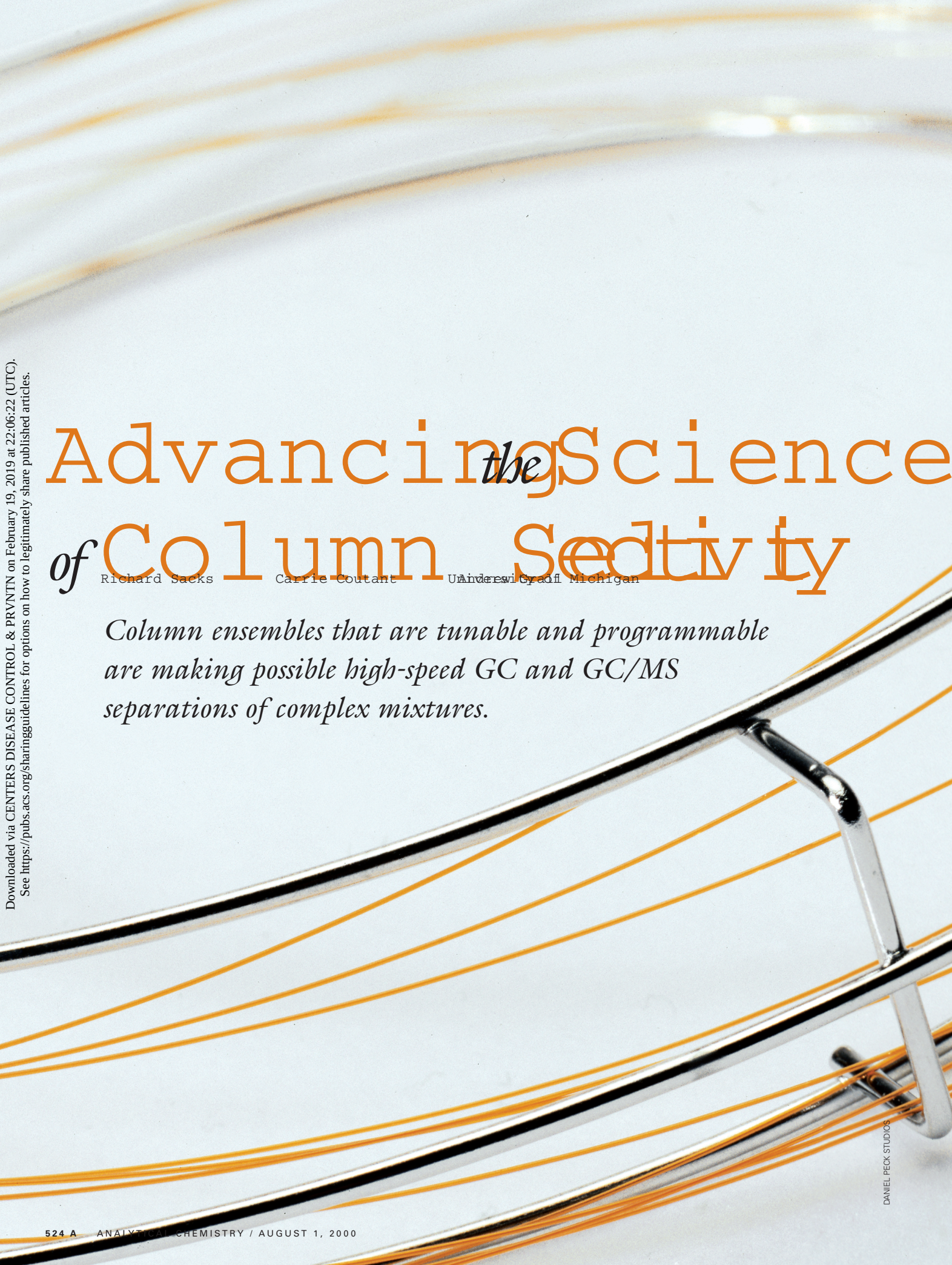
Column ensembles that are tunable and programmable are making possible high-speed GC and GC/MS separations of complex mixtures.



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Selectivity has been described as the forgotten variable in GC (*1*), and for good reason. Hundreds of different stationary phases were in use during GC's heyday in the 1960s and 1970s because of the relatively small peak capacity—number of perfectly spaced peaks in a chromatogram at a specified resolution—associated with packed-column chromatograms. But with the growing acceptance of fused-silica capillary columns with much greater peak capacities, the number of widely used stationary phases declined steadily through the 1980s. Long capillary columns, with their high resolving power, obviated much of the need for highly selective stationary phases. By the mid-1990s, roughly five different stationary-phase chemistries could accomplish more than 90% of all GC separations.

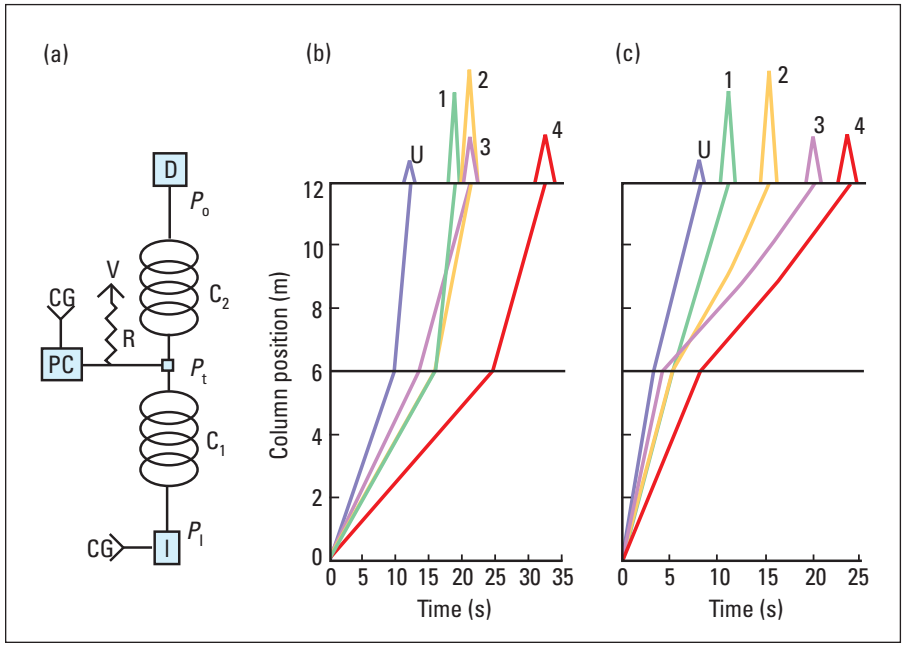


FIGURE 1. Pressure-tunable GC column ensembles.

(a) C_1 and C_2 are capillary columns with different stationary phases; inlet (I); detector (D); electronic pressure controller (PC); carrier-gas inlets (CG); capillary pneumatic restrictor (R); vent point (V); inlet pressure (P_I); outlet pressure (P_o); tuning pressure (P_t). Band trajectory plots are shown for (b) an 80% hold-up-time contribution and for (c) a 40% contribution from C_1. The solid horizontal lines on the band trajectory plots correspond to the column junction point. The band trajectory plots labeled U are for a component that is unretained on either column, and the slopes of these plots represent the carrier-gas velocity profiles in the column ensemble. The retention factors used for the four arbitrary components (1–4) have values of 0.63, 0.62, 0.35, and 1.50, respectively, on C_1 and values of 0.21, 1.12, 2.29, and 2.29, respectively, on C_2. These band trajectory plots are for the case of two 6-m-long, 0.25-mm i.d. columns, using H_2 as the carrier gas. For both columns, the inlet pressure was 28.0 psia, and the outlet pressure was 1 atm.

Investigations of capillary columns in high-speed GC (HSGC) applications began shortly after Golay developed the wall-coated capillary column in 1957 (*2*, *3*). High carrier-gas flow rates could be used with relatively short capillary columns, and resolution could be sacrificed for speed. However, despite a number of important pioneering studies (*4–8*), interest in HSGC has developed slowly. Recently, there has been heightened interest in the technique, and new technologies are rapidly emerging, including special inlet systems, which produce very narrow vapor plugs (*9, 10*); high-speed temperature programming (*11, 12*); microbore capillary columns (*13*); and column ensembles with tunable selectivity (*14, 15*).

The development of time-of-flight (TOF) MS for characterizing peaks from high-speed chromatograms has been concurrent with HSGC advances (*16, 17*). In HSGC, sample component peaks are usually too narrow to be tracked using scanning MS instruments unless relatively few selected ion masses are monitored, but this approach can waste much of the chemical information contained in the mass spectrum. Alternatively, commercial TOF instruments designed for HSGC detection can acquire up to 500 complete mass spectra/s and are equipped with software for automated peak finding, as well as spectral deconvolution of severely overlapping chromatographic peaks (*18*). This often reduces resolution requirements, allowing for even faster analysis and characterization of unknown mixtures.

Because of low peak capacity, HSGC using short capillary columns (1–10 m) has been restricted to analyzing relatively simple mixtures. However, separation times are typically 10–100 times shorter, despite HGSC peak capacities comparable with values obtained using conventional packed columns. Enhanced selectivity, in which peak capacity is used more efficiently, is the key to applying HSGC to more complex mixtures. Manufacturers are beginning to meet this challenge, and some columns are now available that are designed for HSGC applications. These often use mixed stationary-phase functionalities and target certain compounds or compound classes.

A far more general approach is to use column ensembles that have tunable and programmable selectivity (*14, 15, 19–22*) adjusted for specified sets of target compounds. If the compound list changes, selectivity can be altered. In an important series of studies using packed columns, Purnell, Laub, and coworkers showed that mixed stationary phases could be coated on particles, or particles coated with individual stationary phases could be mixed with comparable results (*23, 24*). They also developed procedures for determining the optimal mixing ratio for specified sets of target compounds (*25, 26*). In addition, they showed that similar patterns of eluted peaks could be obtained by coupling two columns using different stationary phases in series and adjusting the length ratio of the columns to achieve a desired selectivity (pattern of eluted peaks) (*27*). However, choosing the best length ratio for the columns was complicated due to carrier-gas compression effects, which result in carrier-gas acceleration from the inlet end to the outlet end of the column ensemble (*28*).

The use of series-coupled ensembles of capillary columns is described in a number of studies aimed at achieving enhanced selectivity with more conventional column lengths and analysis times (*14, 19–22*). Adjusting the column length ratio for selectivity tuning using capillary-column ensembles has been reported (*27*), but a more convenient approach involves controlling the carrier-gas pressure at the junction point of the two columns in the ensemble (*14, 15*). A simple change in junction-point pressure can result in signifi-

cant changes in the pattern of peaks eluting from the ensemble.

If the carrier-gas pressure at the column junction point is changed and the inlet pressure of the first column (injector pressure) and the outlet pressure of the second column (detector pressure) are held constant, the average carrier-gas velocity in the two columns changes differentially—velocity increases in one column and decreases in the other. This results in a differential change in the residence times of all mixture components on the two columns, changing the pattern of peaks eluting from the column ensemble. Independent control of the temperatures of two columns using different stationary phases in a tandem-column ensemble also can enhance selectivity (*29, 30*), but the lack of commercial instruments with two separately controlled ovens limits this approach.

A further complication is that as the number of mixture components increases, the number of possible peak-pair overlaps increases rapidly, and often no single ensemble selectivity will provide large reductions in analysis times. However, if a computer-driven electronic pressure controller is used, computer-selectable elution patterns from the ensemble can be obtained (*31*). Computer-driven systems have been used to separate mixtures containing up to 20 components by HSGC with analysis times of 30–60 s.

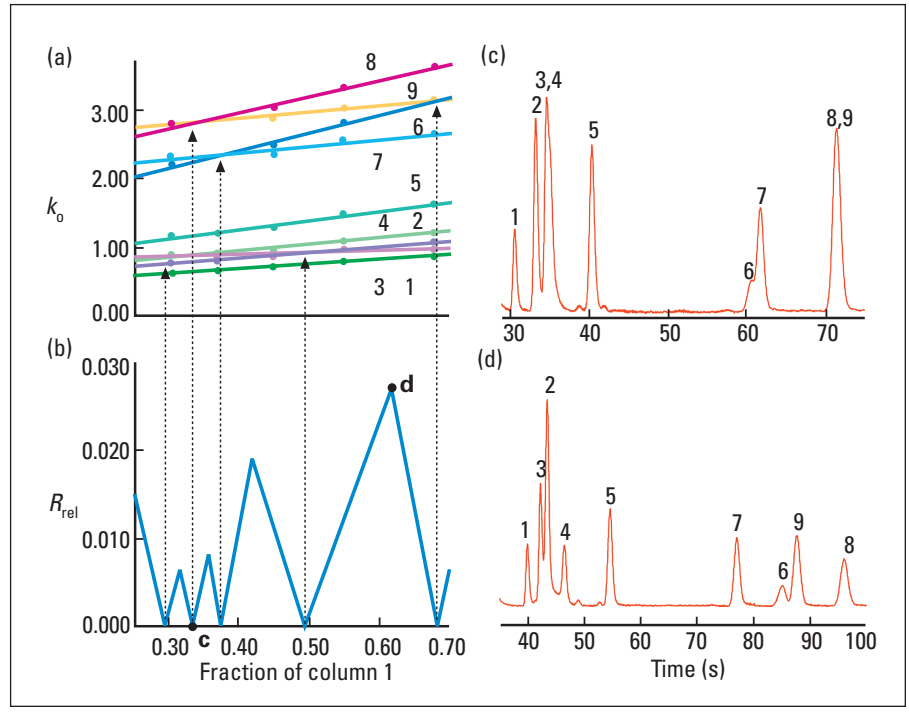


FIGURE 2. Window diagrams can be used to optimize the performance of pressure-tunable column ensembles.

(a) Plots of ensemble retention factor k_o versus the fractional hold-up-time contribution of C_1. The carrier gas was air at an inlet pressure of 1.0 atm, and a photoionization detector was used at a pressure of 0.3 psia (2.1 kPa). The outlet of the vent line also was maintained at 0.3 psia. (b) Window diagram from the plots in (a). Chromatograms corresponding to (c) point c (on the x-axis) and (d) point d (upper right corner) on the window diagram. Peaks are as follows: 1, cyclohexane; 2, cyclohexene; 3, benzene; 4, 2,2,4-trimethylpentane; 5, methylcyclohexane; 6, cycloheptane; 7, toluene; 8, tetrachlorethylene; 9, 2-fluorotoluene.

With computer control of ensemble selectivity, programming can obtain on-the-fly selectivity changes during a separation (*32–34*). With ensemble selectivity programming, the selectivity (junction-point pressure) is initially set to give the fastest separation of the earliest eluting components. To obtain enhanced separation of later-eluting mixture components, selectivity is changed one or more times before the end of the separation.

Pressure-tunable column ensembles

A series-coupled ensemble of two capillary columns using adjustable carrier-gas pressure at the column junction point can be used to tune ensemble selectivity (Figure 1a). Two capillary columns containing different stationary phases are connected to the inlet and detector. For example, a nonpolar polydimethylsiloxane column has been used in combination with a polar polyethylene glycol or a polar trifluoropropylmethyl polysiloxane column (*21, 31, 34*). A computer-driven electronic pressure controller with a carrier-gas supply is connected to the junction point between the columns. A vent line that includes a capillary pneumatic restrictor is also connected to the column junction point. Carrier gas from the controller continuously flows through the vent line, which provides for more rapid pressure equilibration when the set-point pressure is changed and access to lower junction-point pressure values.

The inlet pressure (P_I, head pressure for C_1, the first column) is fixed and is the highest pressure in the system. The outlet pressure (P_o) is also fixed and is the lowest pressure in the system. If a flame ionization detector is used, then P_o is 1 atm. For studies with a TOFMS detector or a closed-cell photoionization detector, P_o is much less than 1 atm. Changes in ensemble selectivity are obtained by changing the tuning pressure (P_t) at the column junction point. If P_t is increased, the pressure drop for the first column decreases, and the drop for the second column increases. This results in a decrease in average carrier-gas velocity (increased hold-up time) for C_1 and an increase in the average gas velocity (decreased hold-up time) for the second column (C_2). The result is increased residence times for all sample components in C_1 and decreased residence times in C_2. The pattern of peaks eluting from the column ensemble depends on the fractions of the ensemble hold-

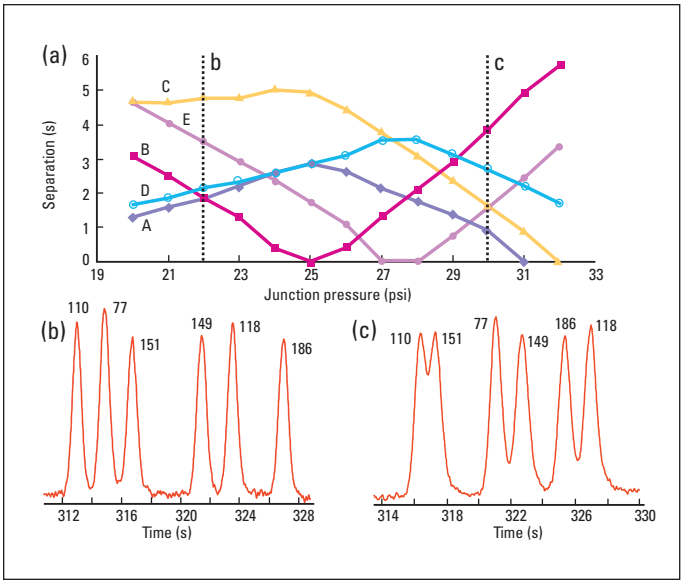


FIGURE 3. Pressure-tunable column ensembles can be optimized for temperature-programmed GC applications.

(a) Plots of nearest-neighbor separation versus column junction-point pressure for six PCB congeners. Chromatograms obtained with junction-point pressures of (b) 22.0 psia and (c) 30.0 psia. Peak numbers correspond to IUPAC congener numbers for PCBs. For (b) and (c), the ensemble temperature was initially set at 70 °C, and a linear temperature ramp with a rate of 30 °C/min was initiated at the time of sample injection. Chromatograms were obtained with a flame ionization detector and an ensemble consisting of a 10-m trifluropropylmethyl polysiloxane column followed by a 10-m polydimethyhylsiloxane column. Both columns have an i.d. of 0.25 mm and a 0.25-μm stationary phase.

up time that can be ascribed to the individual columns. Varying P_t changes these hold-up-time fractions from the two columns and thus changes the ensemble selectivity.

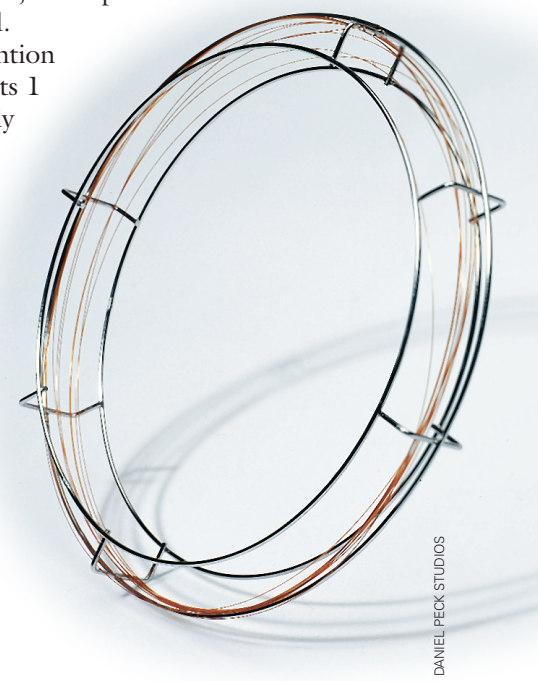
The junction-point P_t can be set anywhere between the head pressure of C_1 (GC inlet pressure) and the vent-point pressure. If the ensemble outlet is operated at subambient pressure, the vent point also can be maintained at subambient pressure to increase the available junction-point pressure range. If the junction-point pressure approaches the GC inlet pressure, the average carrier-gas velocity in C_1 approaches zero. This results in long ensemble hold-up times and poor efficiency for C_1. If the junction-point pressure approaches the detector pressure, the average carrier-gas velocity in C_2 approaches zero with similar results. For the systems described in this article, typically 60–80% of the available pressure range is utilized.

Figures 1b and 1c illustrate the concept of pressure-tunable ensemble selectivity. The positions of the sample bands along the column ensemble versus time are shown as the bands from an arbitrary four-component mixture migrate through the column ensemble. The horizontal lines in the middle of the figures correspond to the column junction point. Sample injection occurs at the lower left corner of each figure, and elution occurs along the top horizontal line. These computer-generated band trajectory plots take into account the acceleration of carrier gas from the highest pressure at the inlet to the lowest pressure at the detector and the change in retention factor for each mixture component as it migrates across the junction from C_1 to C_2. The slopes of these plots give the local band migration velocities. The slight upward curvature observed in the plots is the result of carrier-gas acceleration. The curvature is slight because the inlet-to-detector pressure ratio is relatively small for the total column ensemble length described in this example.

The band trajectory plots in Figure 1b were obtained using a tuning pressure of 25.4 psia. For this pressure, the hold-up time for C_1 is 9.64 s and contributes ~80% to the total hold-up time for the column ensemble. The resulting chromatogram is shown above the band trajectory plots. The junction set-point-pressure in this case is greater than the pressure that would exist in the absence of the pressure controller or any other connection to the junction point. Thus, carrier gas from the controller flows into the column junction point, and this produces an increase in carrier-gas velocity in C_2 relative to the case with no external connections to the column junction point. This is seen as an increase in slope for trajectory U (unretained component) as it crosses the column junction.

For this value of P_t, a complete separation is not obtained. The band trajectory plots in Figure 1c were obtained with a tuning pressure of 20.0 psia. In this case, the C_1 hold-up time is 3.16 s and is 40% of the ensemble hold-up time. This increases the carrier-gas velocity and decreases the component residence times in C_1. Here, carrier gas and sample from C_1 is split between C_2 and the vent line, and the carrier-gas velocity in C_2 is lower than for the case with no external connection to the column junction point. In that case, a complete separation is obtained.

Note that the retention factors for components 1 and 2 on C_1 are nearly the same (0.63 and 0.62, respectively), and thus these components would not be adequately separated using C_1 alone. Also, components 3 and 4 have the same retention factors on C_2 (2.29), and thus would not be separated on C_2 alone. By combining the two



columns, unique selectivity can be achieved—for Figure 1c, this is a complete high-speed separation.

Window-diagram methods

For a tunable column ensemble, the ensemble retention factor k_o for each mixture component can be determined from the ensemble values of retention time t_{Ro} and hold-up time t_{mo}.

$$k_o = (t_{Ro} - t_{mo})/t_{mo} \qquad (1)$$

Ensemble retention factors also can be expressed as the weighted sum of the retention factors k_1 and k_2 for the individual columns comprising the ensemble.

$$k_o = f_1k_1 + f_2k_2 = f_1(k_1 - k_2) + k_2 \qquad (2)$$

in which the weighting factors f_1 and f_2 correspond to the relative contributions that C_1 and C_2 make to the ensemble hold-up time. Values for f_1 and f_2 are calculated from the fractions of the ensemble hold-up time that can be attributed to each of the columns.

$$f_1 = t_{m1}/t_{mo} \text{ and } f_2 = t_{m2}/t_{mo} \qquad (3)$$

Note that $f_1 + f_2 = 1$. Hold-up times t_{m1} and t_{m2} for the individual columns can be calculated from standard equations for gas flow in capillary columns (*35*) or determined empirically by using a second detector connected to the column junction point (*33*).

From Equation 2, plots of k_o versus f_1 should be straight lines with the slope equal to $k_1 - k_2$ and the intercept equal to k_2. Figure 2a shows such plots for a nine-component mixture using a 4.5-m polydimethylsiloxane column followed by a 7.5-m trifluoropropylmethyl polysiloxane column. The ensemble was operated isothermally at 30 °C.

Using air at atmospheric pressure as the carrier gas and a vacuum pump to pull the carrier gas and injected samples through the column ensemble and detector is part of a study aimed at developing small, lightweight GC instruments requiring no on-board carrier-gas tanks. In general, very linear plots are obtained, and extrapolations of the plots to C_1 fraction values of 1.0 and 0.0 give the retention factors for the components on C_1 and C_2, respectively.

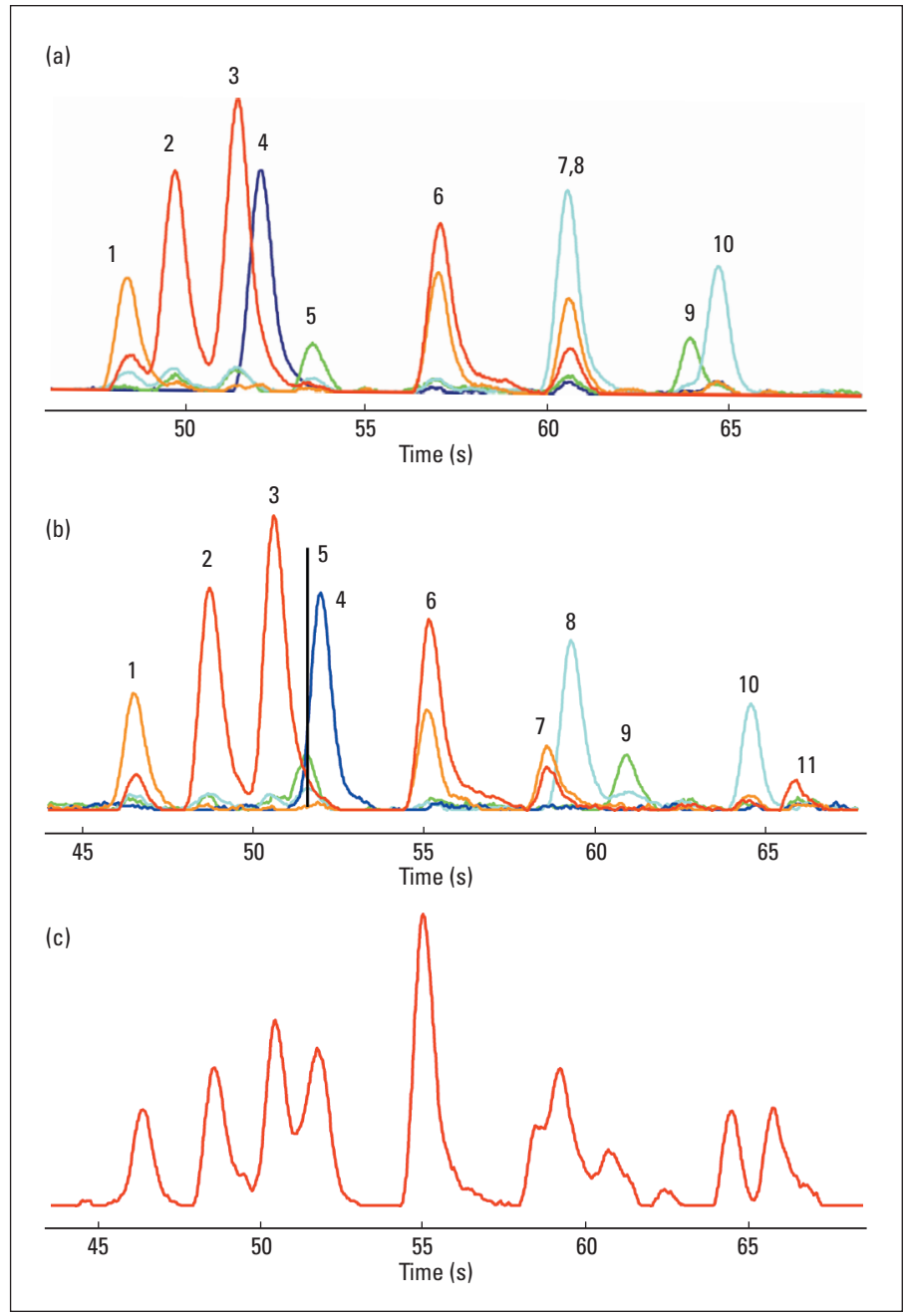


FIGURE 4. High-speed characterization of 11 pesticides can be accomplished using tunable column selectivity with TOFMS detection.

Extracted ion chromatograms for a column junction-point pressure of (a) 42.0 psia and (b) 26.0 psia. (c) Analytical ion chromatogram for a column junction-point pressure of 26.0 psia. Peaks are as follows: 1, heptachlor epoxide; 2, g-chlordane; 3, a-chlordane; 4, 4,4′-DDE; 5, endosulfan I; 6, dieldrin; 7, endrin; 8, 4,4′-DDD; 9, endosulfan II; 10, 4,4′-DDT; 11, endrin aldehyde. The ensemble consisted of an 8.0-m, 0.18-mm i.d. trifluropropylmethyl polysiloxane column followed by a 9.9-m, 0.18-mm i.d. polydimethylsiloxane column. Hydrogen was used as the carrier gas. The initial temperature of the GC oven was 200 °C, and a 70 °C/min linear ramp to 300 °C was initiated at the time of injection. The spectral acquisition rate was 100 spectra/s, requiring a peak apex separation of at least 30 ms for automated peak finding.

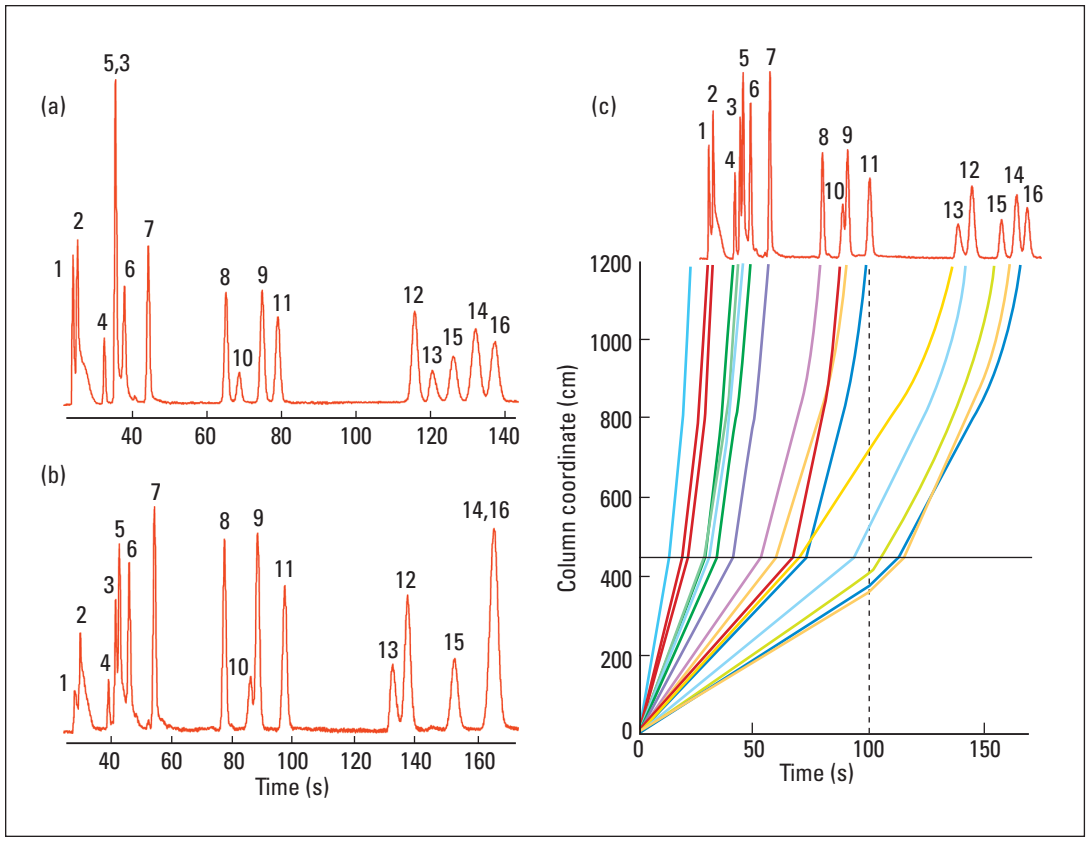


FIGURE 5. Programmable column ensemble selectivity.

Chromatograms obtained at junction-point pressures of (a) 11.5 psia and (b) 12.6 psia. (c) Chromatogram and band trajectory plots for a junction-point pressure change occurring at 100 s after injection (broken vertical line). The solid horizontal line on the band trajectory plot corresponds to the column junction point. Peaks are as follows: 1, trans-1,2-dichloroethylene; 2, 1-hexene; 3, benzene; 4, cylcohexane; 5, cylcohexene; 6, 2,2,4-trimethylpentane; 7, methylcylcohexane; 8, toluene; 9, 2-fluorotoluene; 10, cycloheptane; 11, tetrachloroethylene; 12, chlorobenzene; 13, hexanal; 14, 2,5-dimethyl-2,4-hexadiene; 15, ethylbenzene; 16, *p*-xylene.

For isothermal separations, window-diagram optimization strategies are used to determine the values of f_1 and f_2, which will give the most complete separation (*25, 26, 36*). A window diagram is constructed by computing values for some separation-quality parameters, such as resolution, relative retention, or relative resolution (R_{rel}) for all possible peak pairs, and determining the minimum value for every value of f_1 (*36*).

$$R_{rel} = \Delta k_o/(k_{oa} + 1) \qquad (4)$$

in which Δk_o is the difference in ensemble retention factor values for a component pair, and k_{oa} is their average value.

Figure 2b shows a window diagram obtained from the plots in Figure 2a. For an n-component mixture, there are $(n^2 - n)/2$ unique peak pairs. For each f_1, the peak pair giving the smallest value of R_{rel} defines the most difficult to separate peak pair (critical pair). Note that different peak pairs become the critical pairs for different ranges of f_1. For every f_1 value in which a pair of plots in Figure 2a cross, the corresponding components will coelute, and R_{rel} is 0. Crossing points define the valley points in Figure 2b. The broken vertical lines ending in arrows connect the valley points to the corresponding plots for the critical component pairs in Figure 2a.

The tallest window defines the f_1 value giving the best separation of the worst-case component pair and thus the best overall separation. Figures 2c and 2d show chromatograms obtained for f_1 values corresponding to points labeled c (x-axis) and d (upper right), respectively, in Figure 2b. The top chromatogram was obtained with $f_1 = 0.33$, and several coelutions are observed. Note that this f_1 value corresponds to a valley point in the window diagram. The bottom chromatogram was obtained with $f_1 = 0.62$, corresponding to the peak of the best window, substantially improving the separation.

Selectivity tuning for temperature-programmed GC

Pressure-tunable column ensembles can be temperature-programmed for applications involving mixtures with a wide range of boiling points. However, predicting retention times is more difficult because component bands accelerate along the ensemble axis due to steadily decreasing retention as the ensemble is heated. At sufficiently high-temperature programming rates, the widths of the elution peaks from a single column are nearly constant over a limited range of retention values. This is also true for pressure-tunable column ensembles. Although the elution peak widths vary with column junction-point pressure due to the effects of the carrier-gas velocity on the ensemble efficiency, this variation is relatively small over the range of junction-point pressures.

Because peak widths are relatively constant for the parameter values used in this study, window diagrams can be constructed by plotting the separations of all adjacent peak pairs

versus the junction-point pressure. Such a plot is shown in Figure 3a for six polychlorinated biphenyl (PCB) congeners, which elute in the same general region of a temperature-programmed separation. Chromatograms obtained at two different ensemble selectivities (junction-point pressure values) are shown in Figures 3b and 3c.

For these six components, there are five adjacent peak pairs. The peak pairs change as the elution order changes with junction-point pressure. Note that the elution order is very different in the two chromatograms. Plot A in Figure 3a shows the separation of the first two peaks in the chromatogram as a function of pressure. Note that congeners 110 and 77 are the first peak pair for pressures of 20–25 psia. For pressure >25 psia, the elution order changes and congeners 110 and 151 are the first component pair. At 31 psia, congener pair 110–151 coelutes, and the separation in A goes to zero. Plot B gives the separation of the second and third peaks to elute. This second peak pair consists of congeners 77 and 151 over the entire junction-pressure range. However, their elution order reverses at 25 psia, where their separation goes to zero.

The two most favorable windows in Figure 3a occur at junction-point pressure values of 22 psia, where plots A and B intersect, and at ~29.5 psia, where plots A and E intersect. The broken vertical lines at junction pressure values of 22.0 and 30.0 psia give the adjacent peak-pair separations for Figures 3b and 3c, respectively. As expected from the window diagram, the chromatogram obtained at a junction-point pressure of 22.0 psia has a greater critical-pair separation than the chromatogram obtained at 30.0 psia.

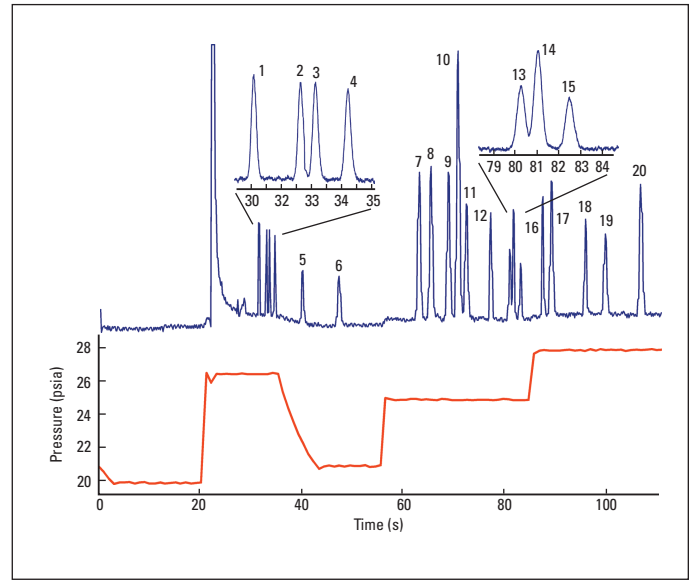


FIGURE 6. High-speed separation of 20 pesticides using pressure-programmable selectivity with a multistep pressure program.

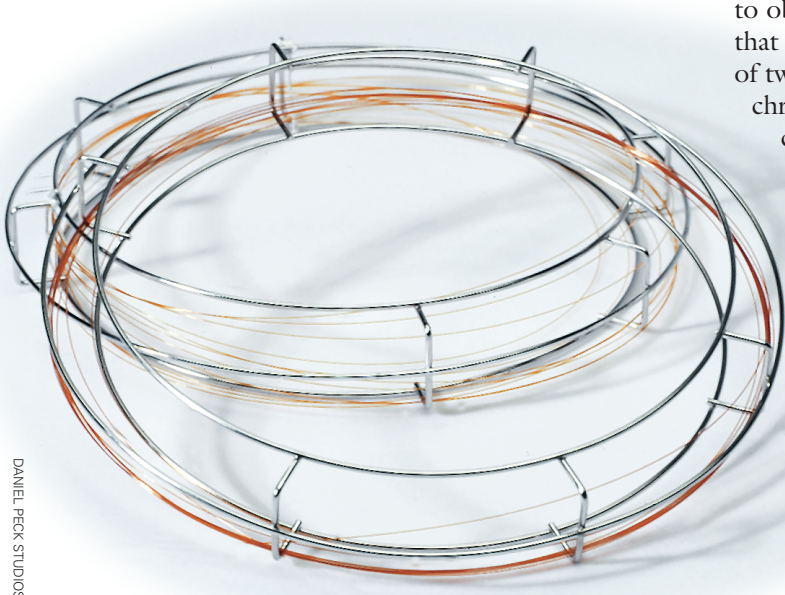
Insets show congested regions on an expanded time scale. The pressure program is shown below the chromatogram. Peaks are as follows: 1, a-BHC; 2, g-BHC; 3, b-BHC; 4, D-BHC; 5, heptachlor; 6, aldrin; 7, heptachlor epoxide; 8, g-chlordane; 9, a-chlordane; 10, 4, 4,4′-DDE; 11, endosulfan I; 12, dieldrin; 13, endrin; 14, 4,4′-DDD; 15, endosulfan II; 16, 4,4′-DDT; 17, endrin aldehyde; 18, methoxychlor; 19, endosulfan sulfate; 20, endrin ketone.

Column ensembles for HSGC/TOFMS

If a TOFMS instrument has time-array detection (*16, 17*), complete sample fragmentation patterns from electron-bombardment ionization can be acquired at rates up to 500 spectra/s. The instrument's software uses automated peak finding and, if the fragmentation patterns are sufficiently different, can deconvolute and characterize severely overlapping chromatographic peaks from unknown components.

Because resolution requirements are greatly relaxed for many peak pairs, the time axis of chromatograms can be compressed by using shorter columns, higher oven temperatures, or higher temperature programming rates, resulting in very rapid analysis and characterization of unknown mixtures. However, the peak apex separation must be sufficient to obtain two complete mass spectra between the apexes so that the peak-finding algorithm can recognize the presence of two or more peaks for unknown components in a single chromatographic feature. In this case, pressure-tunable column ensembles are useful for structuring the chromatogram to take full advantage of the automated peak-finding and spectral deconvolution features.

Figure 4 shows the separation and characterization of a mixture containing 11 common pesticides. Extracted ion chromatograms for several characteristic ions are shown. Figure 4a was obtained with a junction-point pressure of 42 psia corresponding to a fractional hold-up-time contribution from the first column of ~0.63. The separation of components 7 and 8 was <30 ms, and only a single peak was found by the automated peak-finding software. While components 3 and 4 show considerable overlap, both components were



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found and correctly identified. Under these conditions, component 11 eluted after the end of the time window shown in the figure.

For Figure 4b, the junction-point pressure was changed to 26.0 psia, corresponding to a 0.32 fractional hold-up-time contribution from the first column. Several significant changes in the elution patterns are observed. The elution order of peaks 4 and 5 has reversed, peaks 7 and 8 are now adequately separated for automated peak finding, the separation of peaks 9 and 10 is significantly larger, and peak 11 now elutes just after peak 10. Under these conditions, all 11 component peaks were found and successfully characterized. Note that peak 5 was buried under peaks 3 and 4, but because peak apex separations were adequate, all the peaks were found.

Computer-controlled column selectivity allows for on-the-fly selectivity changes.

Figure 4c shows the analytical ion chromatogram for the same conditions used in Figure 4b. The analytical ion chromatogram is obtained as the sum of all single-ion chromatograms, which exceed a software-selectable S/N threshold, for all found peaks. The analytical ion chromatogram provides information similar to a total-ion chromatogram, but with appreciably larger S/N. It is clear from the analytical ion chromatogram that the chromatographic resolution is completely inadequate for the analysis of this mixture using a single-channel measurement technique—component 5 would be completely overlooked. However, with the combination of pressure-tunable column selectivity to structure the chromatogram and TOFMS to spectrally deconvolute overlapping chromatographic peaks, the complete characterization and analysis of all 11 components was accomplished in a 20-s window with a total analysis time of <70 s.

Programmable selectivity

As the number of components in a mixture increases, it is less likely that any single selectivity will provide a large decrease in separation time. In these cases, programmable selectivity, in which the ensemble junction-point pressure is changed on the fly during a separation, is very useful. The isothermal chromatograms in Figures 5a and 5b were obtained with junction-point pressures of 11.5 and 12.6 psia, respectively, using the same column ensemble and instrument used for Figure 2. Note that for Figure 5a, components 3 and 5 completely coelute. For Figure 5b, the separation of components 3 and 5 is significantly improved, but components 14 and 16 almost completely coelute.

In Figure 5c, band trajectory plots and the corresponding chromatogram are shown for the case when the junction-point pressure is initially set at 12.6 psia to give adequate separation of components 3 and 5. After 100 s, the junction-point pressure is changed on the fly to 11.5 psia, indicated by the broken vertical line on the band trajectory plots. The solid horizontal line at a column coordinate of 450 cm corresponds to the column junction point.

The decreased junction-point pressure after 100 s results in an increase in the carrier-gas velocity in C_1 and a decrease in the velocity in C_2. The velocity increase in C_1 is easily seen from the increase in the band trajectory slopes for components 14, 15, and 16, which are still in C_1 at the time of the pressure change. The plots for components 12 and 13, which are in C_2 at the time of the pressure change, show a small decrease in slope. Components 1–11 have eluted from the column ensemble before the pressure change and thus are unaffected by it.

The separation obtained with selectivity programming was more complete than could be obtained with any single selectivity in a comparable time frame using this specific column ensemble. Note that, while components 12–16 are completely separated with the selectivity program, the elution order of 12 and 13 is reversed from that observed in chromatogram (a). This reversal is due to the influence of the initial selectivity for the first 100 s of the separation when the junction-point pressure was 12.6 psia.

In some applications, multistep selectivity programs can be very useful. Figure 6 shows the temperature-programmed separation of a mixture containing 20 common pesticides. The column temperature was initially set at 200 °C, and a 30 °C/min temperature ramp was initiated at the time of injection. A multistep selectivity program was used to obtain a complete separation in <2 min. The insets show congested portions of the chromatogram on expanded time scales. Components 13 and 14 are the critical pair, and they are separated with a resolution of ~1.1. Using a single, commercially available column designed specifically for this mixture, 20–30 min is required for a separation with a comparable critical-pair resolution.

The pressure program used for the separation is shown below the chromatogram. Note that every pressure change results in an ensemble selectivity change. The relatively slow downward pressure change occurring at 35–42 s is the result of dead volume in the pressure controller (*33*). The program was determined by using plots similar to those in Figure 3a for several different portions of the chromatogram. The last pressure step, occurring ~82 s after injection, does not improve the quality of the separation, but it does drive components 16–20 from C_2 more quickly, resulting in a small but significant reduction in separation time.

Under temperature-programmed conditions, the higher-

boiling-point components are nearly frozen at the inlet end of the first column during the early (low-temperature) portion of the separation. Thus, junction-point pressure changes made early in the separation have little impact on the band trajectories for the higher-boiling-point compounds. In addition, the selection of junction-point pressures for specified component subgroups of the complete mixture can be made more or less independently. This significantly simplifies the optimization procedure.

Technological advantages

Although chromatographic analysis of complex mixtures of organic compounds can be a daunting task, a serially linked pair of capillary separation columns and a computer-driven pressure controller positioned at the junction point of the two columns in the ensemble offer a possible approach to this difficult problem.

As noted, this technology has several desirable characteristics. Using an electronic pressure controller facilitates selectivity tuning with high resolution and repeatability. A wide range of retention patterns can be achieved if the stationary-phase chemistries for the two phases used in the column ensemble are very different, as is the case for nonpolar silicone-based phases and very polar polyethylene glycol. Moreover, computer-controlled column junction-point pressure allows for on-the-fly selectivity changes—deliberate, controlled variations of the ensemble junction-point pressure during the progress of a separation. The use of multistep selectivity programs with temperature-programmed GC should be particularly helpful for the high-speed separation of mixtures that span a wide boiling-point range.

Ongoing and future work should lead to the development of programs for mixtures of interest in environmental, manufacturing, and general laboratory applications. The use of multistep selectivity programming with TOF detection is also under development and will provide unparalleled power for the high-speed analysis and characterization of complex mixtures of organic compounds.

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