



Connections

Peak retention times depend upon relationships between pressure, flow rate, oven temperature, column dimensions, and stationary phase. Analysts cannot control column manufacturing variability, but they can influence gas chromatograph parameters and take steps to maximize column lifetime. Instrument-to-instrument variability can be brought under control by implementing a few simple calibration and setup procedures. In this first of a two-part installment, John Hinshaw discusses the relative effects that inlet pressures and oven temperatures have on capillary gas chromatography.

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Flow, Pressure, and Temperature Calibration: Part I

Laboratories with multiple gas chromatographs that analyze a large number of samples might want to perform a specific analysis on any suitable instrument that is available. For example, if three instruments are equipped with similar autosampler, pneumatic, inlet, oven, and detector options on either the first or second channel, then, in principle, a particular analysis could be performed on any of the instruments upon installation and checkout of the appropriate column. While experienced chromatographers would not expect peak retention times, peak shapes, and peak areas to be exactly the same no matter which gas chromatograph they used, they will have an expectation that running a method on a particular instrument chosen from among equivalent instruments will produce similar results. Along with that expectation, they probably will want to minimize the degree of variation between such instruments or at least understand the reasons for a relative lack of consistency. The degree of similarity between duplicate analyses performed on different instruments depends upon the interinstrument variability of temperature and pressure conditions as well as the columns. Gas chromatography (GC) practitioners can minimize these effects by taking steps to ensure that the conditions for an analysis are as consistent as possible from one instrument to another and by taking good care of their columns.

Statistics tells us that we cannot expect instrument-to-instrument variability to be any better than the variability of each instrument operating alone. Experience tells us that, in fact, it usually is worse. In addition, an instrument might be capable of delivering excellent repeatability over the course of 10 consecutive runs made one immediately after another, yet the same instrument probably will not deliver the same average retention times when restored to running one method after being used for some time for another analysis.

Analysts can influence the variability of individual instruments by following good laboratory practices to set up, operate, and

maintain them. Carrier-gas leaks, exhausted filters, poor quality septa, sloppy column installation, degraded columns, and incorrect detector flows — all among the many opportunities for introducing errors — will contribute extra variability from run to run, and thus from instrument to instrument. Periodic performance checks with a test sample can help flag an instrument that needs additional attention and troubleshooting.

Chromatographers should set realistic expectations for the performance of different instruments in relation to each other. Each instrument and method combination has a minimum level of run-to-run variability: better performance cannot be expected on that instrument with that specific methodology. There also exists an absolute best performance level determined by the instrument design itself, which is seldom attained with real-world samples and conditions but might be achieved with a test sample under ideal conditions. Reasonable expectations for acceptable results should be somewhat worse than the best performance expected for the specific method and instrument, but not so much worse that useful sample information would be lost when that worst performance level is approached.

Method validation and check samples ensure that a specific instrument, column, and data-handling system is capable of delivering desired minimum performance levels. By performing regular flow, pressure, and temperature calibration, analysts help ensure successful validation while enabling their instruments to produce more consistent results. Instrument calibration (as opposed to method calibration for quantitation) is not a substitute for validation but rather an adjunct that improves the overall quality of results.

In addition to temperature and pressure, flowrates also are important. I do not usually recommend direct measurement of capillary column flows due to the very low rates less than 3 mL/min and the high diffusion of helium. Instead, electronic pressure control systems can compute the flows

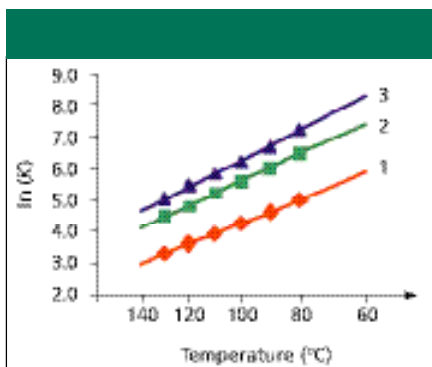


Figure 1: Data points and regression lines for *n*-hydrocarbons at differing oven temperatures. (1) *n*-nonane; (2) *n*-undecane; and (3) *n*-dodecane. Column: 30 m × 0.53 mm i.d. × 3.0- μ m 5%-phenyl-95%-methylsilicone; carrier: He at 91 cm/s. The temperature scale is in reciprocal Kelvin degrees but is labeled with the equivalent centigrade temperature for clarity.

with sufficient accuracy or the operator can calculate flow rates from the linear velocity and column dimensions. Split flows, on the other hand, are measured easily and have a strong effect on quantitation. They, as well as detector flow rates, should be measured and verified independently as part of normal laboratory quality assurance, even though built-in flow controllers appear to operate correctly.

The first part of this two-part article will focus on the effects of chromatography variables on peak retention times in the context of transferring existing methods from one instrument to another. The second installment will investigate questions related to instrument temperature, pressure, and flow calibration with a goal of minimizing inter-instrument variations.

The Effect of Chromatography Variables on Retention Times

Retention times in GC are controlled by a number of interconnected physicochemical relationships. Within these are a number of primary variables over which chromatographers have some influence, such as the temperature, pressure, and column dimensions. Before delving into how these variables directly affect the chromatography, however, I'll quickly review some of their relationships in order to establish a forum for the discussion. Readers who are familiar with this theoretical material can skip to the next section. I'll base the retention modeling on some data for normal hydrocarbons from an earlier "GC Connections" installment (1). GC theory predicts real-world behavior closely enough to allow us to use theory as a surrogate when having a

discussion of this nature.

The primary variables encapsulated by these relationships can be divided into two classes: variables related to the column and those related to the instrument. The assumption that these two variable sets are independent is generally valid, as changing temperature does not change the column diameter, for example, and installing columns of differing lengths will not affect the inlet pressure. Selecting the setpoints of the independent variables determines the values of other quantities that depend upon them.

The familiar equation that relates retention time t_R to retention factor k and unretained peak time t_M , shown in equation 1, is a good starting point for the discussion.

$$t_R = t_M \cdot (k + 1) \quad [1]$$

This equation says that the retention time depends only upon the unretained peak time and the retention factor. For the purposes of this discussion, equation 1 separates control of the retention time into two categories: those factors that affect the unretained peak time and those that affect the retention factor. This division lands instrument and column dimension factors in the first category and column chemistry factors into the second. It is the instrument parameters that are under examination here, primarily the inlet pressure and the oven temperature. Of course, the column temperature also influences stationary-phase solute interactions, and so we will consider its influence in both arenas. Some of the pressure variables usually considered as independent (such as the column pressure drop) actually are dependent upon other, more fundamental variables such as the absolute column inlet and outlet pressures.

Table I: Result of log-linear regression for the partition coefficient of three hydrocarbon peaks over a range of temperatures. *A* and *B* are the coefficients in equation 9; *r* is the regression coefficient. For conditions, see Figure 1

Peak	<i>A</i>	<i>B</i>	<i>r</i>
<i>n</i> -C ₉	4946	8.99	0.999967
<i>n</i> -C ₁₁	5717	9.72	0.999913
<i>n</i> -C ₁₂	6205	10.37	0.999716

However, for practical reasons, we will work with the parameters that chromatographers normally set and read, namely, the inlet gauge pressure and the column temperature setpoint.

Influence of Pressure and Temperature

Unretained peak times are influenced by the average carrier gas linear velocity \bar{u} and the column length *L*:

$$t_M = \frac{L}{\bar{u}} \quad [2]$$

The average carrier gas velocity is in turn a function of the carrier gas viscosity η , the pressure drop across the column Δp , and the column diameter d_c :

$$\bar{u} = \frac{\Delta p \cdot j' \cdot d_c^2}{32L \cdot \eta} \quad [3]$$

The average linear velocity is proportional to the pressure drop across the column, tempered by a pressure correction factor j' as well as to the reciprocal of the carrier-gas viscosity. The viscosity is influenced by temperature (see equation 6), but that influence is small across the narrow temperature range encountered between instruments set to the same nominal temperature.

Pressure: The column pressure drop, which outside of mass spectrometric or

Table II: Retention time for three peaks as a function of column temperature operated at a constant inlet pressure of 15 psig. For conditions, see Figure 2

Temperature (°C)	Peak Retention Time (min)		
	<i>n</i> -Dodecane $K_{100\text{ }^\circ\text{C}} = 522.1$	<i>n</i> -Undecane $K_{100\text{ }^\circ\text{C}} = 270.2$	<i>n</i> -Nonane $K_{100\text{ }^\circ\text{C}} = 70.99$
60	18.68	8.89	2.73
70	11.55	5.96	2.21
80	7.53	4.23	1.90
90	5.19	3.19	1.70
100	3.79	2.55	1.58
110	2.94	2.15	1.50
120	2.41	1.90	1.45
130	2.07	1.74	1.43
140	1.86	1.63	1.41

atomic emission detectors is equal to the gauge pressure, directly influences retention times. Higher pressures produce higher velocities, and in turn, shorter retention times. Differences in the pressures actually delivered to the column will give rise to differences in observed retention times between instruments.

The pressure correction factor relates the relative pressure across the column to the average velocity as follows:

$$f = \frac{3}{4} \cdot \frac{(P^2 - 1)(P + 1)}{P^2 - 1} \quad [4]$$

where P is the relative pressure across the column:

$$P = \frac{P_1}{P_0} \quad [5]$$

This correction factor has a minor influence on average velocities. It ranges from 1.0 down to about 0.82, with the column outlet at atmospheric pressure and inlet pressures ranging from 1 to 140 psig. At low outlet pressures such as encountered with a direct column interface to a mass spectrometer, f approaches 0.75. I have included it only because it is easy to calculate with a spreadsheet, and it gives a more accurate characterization of the pressure-retention time relationship.

Viscosity: The carrier gas viscosity itself is a function of the type of carrier gas and the column temperature according to this empirical function (2):

$$\eta(T) = \eta_0 \left(\frac{T}{T_0} \right)^x \quad [6]$$

where T is the current temperature, T_0 is the reference temperature (usually 0 °C), η_0 is the viscosity at the reference tempera-

ture, and x is an exponent that depends upon the carrier gas. For helium, $x = 0.646$ and $\eta_0 = 18.662 \times 10^{-6}$ Pa·s. Note that equation 6 is derived by curve fitting to measured viscosities from a variety of sources. This equation, or one very similar to it, is used in electronic pressure control systems to compensate for the effects of column oven-temperature changes on the average linear carrier-gas velocity and the column flow rate.

As temperature increases, so does the viscosity. The temperature effect is large enough to increase the viscosity by a factor of 1.5 when heating from 50 °C to 350 °C. In turn, at a constant inlet pressure, the average carrier-gas velocity will decrease proportionately at higher temperatures, and the column flow rate will decrease by a similar factor. Electronic pressure control compensates for these viscosity changes and will keep the average linear velocity or the column flow rate constant by increasing the inlet pressure as required by higher column temperatures, canceling out the viscosity effect.

A constant column flow rate is important when temperature programming the column oven with flow-sensitive devices such as thermal conductivity detectors or mass spectrometric detectors, and it also helps reduce retention times at high column temperatures. The effect of carrier-gas viscosity is significant over a wide temperature range such as encountered in column oven-temperature programming, but it can be ignored for small temperature shifts such as encountered when moving methods from one instrument to the next.

Phase ratio: Temperature also influences the retention factor as established by its relationship with the partition coefficient K and the phase ratio β :

$$k = \frac{K}{\beta} \quad [7]$$

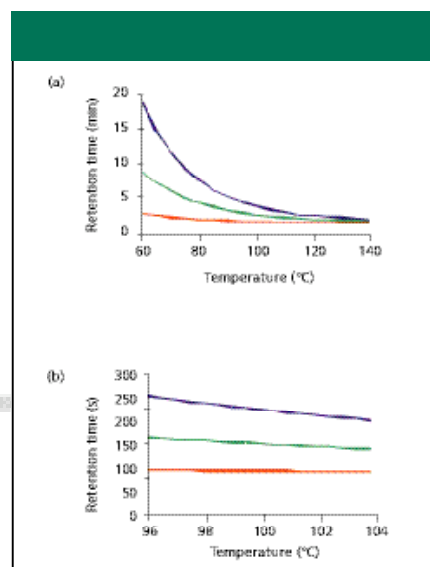


Figure 2: Effect of temperature on retention times. (a) Column temperature = 60–140 °C; (b) Close-up view, column temperature = 96–104 °C. Column: 25 m × 0.25 mm; inlet gauge pressure: 15 psig He (103 kPa); column outlet at room pressure; $u = 33$ cm/s. Key: n-dodecane, $K_{100\text{ °C}} = 522.1$ (blue); n-undecane, $K_{100\text{ °C}} = 270.2$ (green); n-nonane, $K_{100\text{ °C}} = 70.99$ (red).

The phase ratio (the ratio of the gas-phase volume to the liquid-phase volume in the column) is temperature-independent. It is determined by the column diameter and film thickness:

$$\beta = \frac{d_c}{4d_f} \quad [8]$$

A 0.25 mm i.d. × 0.25 μm film column has a phase ratio of 250, while the same column with a 5- μm film has a phase ratio of 12.5, which yields partition coefficients that are 20 times greater than for the 0.25- μm film column. This means that, as anyone who has tried it knows, the thicker film column will produce proportionately longer retention times. Short of the wholesale removal of the stationary phase through mistreatment of the column, the phase ratio can be considered a constant for any individual column. Over time, the phase ratio might decrease somewhat through gradual phase loss at higher temperatures or by column baking or rinsing. Such losses will reduce the retention factors correspondingly, and they can be tracked over time by validation procedures.

Partition coefficient: In the upper half of equation 7, the partition coefficient K expresses the ratio of the stationary phase concentration of a solute to its mobile phase concentration at a particular temper-

Table III: Retention time for three peaks across a narrow temperature range operated at a constant inlet pressure of 15 psig. For conditions, see Figure 2

Temperature (°C)	Peak Retention Time (s)		
	n-Dodecane $K_{100\text{ °C}} = 522.1$	n-Undecane $K_{100\text{ °C}} = 270.2$	n-Nonane $K_{100\text{ °C}} = 70.99$
96	255.9	166.4	97.1
97	248.3	162.8	96.4
98	241.0	159.5	95.7
99	234.0	156.2	95.1
100	227.4	153.2	94.5
101	221.1	150.2	94.0
102	215.1	147.4	93.4
103	209.4	144.8	92.9
104	203.9	142.2	92.4

ature. Higher K values mean that a solute will take longer to be eluted. Temperature strongly influences the value of K . As temperature increases, K decreases according to this generalized relationship:

$$K = e^{\frac{A}{T} + B} \quad [9]$$

where A and B are arbitrary constants related to the chemical interactions between a specific solute and stationary phase. For GC separation modeling by computer, the values of A and B can be determined from a few retention time measurements at different temperatures. Their values are not the same for different solutes on the same stationary phase nor for the same solutes on different phases. However, the coefficients are independent of the column dimensions, film thickness, and carrier gas flow with isothermal elution.

A long while back, I had measured the retention behavior of a number of test solutes over a range of column temperatures for a "GC Connections" installment in 1992 (1). I dug up the original chromatograms from an archival CD-ROM and copied the retention times and unretained peak times for each temperature into a spreadsheet. Then I calculated the peaks' retention factors k by rearranging equation 1, and I found their partition coefficients K using equation 7 with the phase ratio of the 0.530 mm i.d. \times 3.0 μ m film column used to acquire the original chromatograms, which according to equation 8 was equal to 44.2. For each of the three selected hydrocarbon peaks, I performed linear regression on the natural logs of the partition coefficients against the reciprocal of the absolute oven temperature, the results of which are shown in Table I. These constants form the model for evaluation of the effect of temperature and pressure on solute retention. Figure 1 shows the measured data points and the fitted regression lines for the three components as plots of the natural log of the partition coefficients against the reciprocal temperature. Each of the solutes followed equation 9 closely.

As we will see, the influence of column temperature on K that dominates the dependence of retention times on shifting instrument-to-instrument temperatures. Inlet pressure variations also contribute significantly to the variations.

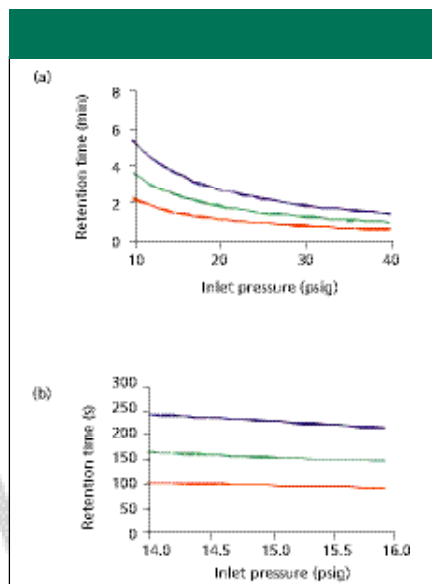


Figure 3: Effect of pressure on retention times. (a) Inlet pressure = 10–40 psig and (b) close-up view, inlet pressure = 14–16 psig. Column: 25 m \times 0.25 mm; column temperature: 100 °C; column outlet at room pressure. Key: *n*-dodecane, $K_{100\text{ }^\circ\text{C}} = 522.1$ (blue); *n*-undecane, $K_{100\text{ }^\circ\text{C}} = 270.2$ (green); *n*-nonane, $K_{100\text{ }^\circ\text{C}} = 70.99$ (red).

Great Expectations

With these relationships on hand, we can start to explore the effects of variability in pressure and temperature on retention times. Analysts expect great results from their instruments, as they should. Under good circumstances, with a single instrument, gas chromatographers might expect to see capillary column isothermal retention time standard deviations of around 1.0 s, or about 0.2% relative standard deviation (RSD) for 10 or more consecutive injections of a peak that is isothermally eluted in 10 min. Such deviations might be smaller or larger, but this level of repeatability should be easy to achieve with an autosampler. Attaining this level of performance presupposes that the oven temperature and column pressure are well controlled (that is, close to the degree for which the manufacturer designed them) and that there are no overriding influences such as peak overload-

ing, leaks, or widely varying split flow rates.

Having set a standard for retention time repeatability, how do variations that are induced by instrument-to-instrument uncertainties in the oven temperature and the inlet pressure compare?

Temperature Effects

First, let us look at the effect that changing the oven temperature across a wide range, as well as across a narrow range, has on retention times. By putting equations 2–8 to work and imposing the results on equation 1, we can compute the temperature effects. This estimate includes the effect of the column temperature on the partition coefficients as well as the additional effect caused by the temperature-related shift in carrier gas viscosity. However, I don't have the original column used to acquire the chromatograms in 1991. Instead, I have simulated the results on a different 25 m \times 0.25 mm column with a 0.25- μ m film thickness with the same type stationary phase. I know the partition coefficients as a function of temperature, so I can use equations 1–9 to estimate retention times on this new column as the temperature and pressure change.

I checked the predicted values for the average linear carrier gas velocity at several different temperatures and pressures using a gas chromatograph with electronic pressure control. I entered the column dimensions into the instrument and set the carrier gas type to helium. Then I set pressures of 15 and 25 psig and temperatures of 50 and 100 °C. In each case, the spreadsheet numbers varied from the GC readouts by much less than one percent.

Figure 2 illustrates what happens to the isothermal retention times of the three hydrocarbon peaks as the temperature shifts. In Figure 2a, the temperature varies across a wide range, from 60–140 °C. Retention times decreased by factors of 1.5–2.5 going from 60 °C and

Table IV: Retention times for three peaks across a wide range of pressures, operated isothermally at 100 °C. For conditions, see Figure 3

Gauge Pressure (psig)	Peak Retention Time (min)		
	<i>n</i> -Dodecane $K_{100\text{ }^\circ\text{C}} = 522.1$	<i>n</i> -Undecane $K_{100\text{ }^\circ\text{C}} = 270.2$	<i>n</i> -Nonane $K_{100\text{ }^\circ\text{C}} = 70.99$
10.0	5.59	3.77	2.32
15.0	3.79	2.55	1.57
20.0	2.89	1.94	1.20
25.0	2.34	1.58	0.97
30.0	1.98	1.33	0.82
35.0	1.72	1.16	0.71
40.0	1.52	1.02	0.63

Table V: Retention times for three peaks across a narrow range of pressures, operated isothermally at 100 °C. For conditions, see Figure 3

Gauge Pressure (psig)	Peak Retention Time (s)		
	<i>n</i> -Dodecane	<i>n</i> -Undecane	<i>n</i> -Nonane
	$K_{100\text{ °C}} = 522.1$	$K_{100\text{ °C}} = 270.2$	$K_{100\text{ °C}} = 70.99$
14.0	242.7	163.5	100.9
14.2	239.4	161.3	99.5
14.4	236.3	159.2	98.2
14.6	233.2	157.1	96.9
14.8	230.2	155.1	95.7
15.0	227.2	153.1	94.5
15.2	224.4	151.2	93.3
15.4	221.6	149.3	92.1
15.6	218.9	147.5	91.0
15.8	216.3	145.7	89.9
16.0	213.7	144.0	88.9

by additional factors of 1.2–2 going from 100 °C to 140 °C, as shown in Table II.

In Figure 2b, the temperature varies across a much narrower range, from 96 °C to 104 °C, which is meant to represent the maximum extent of the oven temperature variability that can be expected going from one instrument to another. As shown in Table III, in going only from 99 °C to 101 °C the resulting retention times vary from 1.2 s for the less retained nonane peak up to as much as 13 s for the more retained dodecane peak. Depending upon the solute retention then, the sensitivity of retention times on temperature ranges from 0.6 s/°C to 6.5 s/°C in this case. More highly retained solutes will experience even larger temperature dependencies. Or to put it another way, retention times can shift up to 15 s or more for instrument-to-instrument temperature variations of ± 1 °C.

Pressure Effects

The effects of pressure shifts are similar to effects due to temperature shifts. Figure 3a illustrates what happens to retention times for the three example solutes when the inlet gauge pressure increases from 10 to 40 psig while holding the oven temperature constant at 100 °C: each peak's retention times are reduced by a factor of ~ 3.7 . This pressure change constitutes an increase in the average carrier gas linear velocity from 23 cm/s to 84 cm/s, which is in the same 1:3.7 proportion. Retention times decrease nearly as the reciprocal of increasing inlet pressure. The data is given in Table IV.

Figure 3b and Table V illustrate the effect on retention times of much smaller shifts in inlet pressure, from 14 to 16 psig. Retention time shifts are significant even when as small as 1 psi, for example from 14.5 to 15.5 psig; the dodecane peak moves in by 14.7 s, the undecane peak by 9.8 s, and the

nonane peak by 6.0 s. These changes are on the same order as would be caused by temperature shifts of about 1 °C, and they will be amplified similarly at longer retention times.

Conclusion

Analysts who must use different gas chromatographs to perform the same methods should be concerned about variability of results from one instrument to the next. Although not a substitute for validation and suitability testing, instrument calibration can help to reduce the variability. The dependency of peak retention times on oven temperature and inlet pressure can be large enough to cause significant deviations of 15 s or more between instruments when the individual oven temperatures differ by only 1 °C or the pressures by 1 psi.

The second installment in this series will discuss instrument temperature, pressure, and flow calibration, as well as the role of column variability in terms of interinstrument performance.

References

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- (2) J.V. Hinshaw and L.S. Ettre, *Introduction to Open-Tubular Column Gas Chromatography* (Advanstar, Cleveland, Ohio, 1994), p. 25.

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