Unlocking Retention Times in Gas Chromatography

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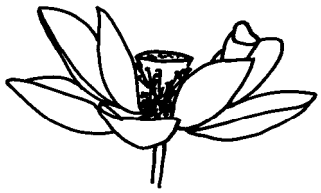

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Time that a molecule spends migrating down a chromatographic column – labeled as retention time – is the primary means of identifying that compound in a mixture. With prudent choices of chromatographic conditions, this chemical can be fully separated from its companions, and then its retention time uniquely identifies its presence. This time is a function of a number of variables, including:

- **choice of column stationary phase,**
- **thickness of the phase,**
- **internal diameter of the column and its length,**
- **carrier flow rate through the column,**
- **column temperature,**
- **sample loading on column, and**
- **choice of carrier gas.**

Some of these have very significant impact on retention of the molecule, while others have more subtle, but important effects.

Peak Identification by Retention Time and Spectroscopy

The classical chromatographic process involves injecting a standard of known composition to determine retention times of the desired analytes. A peak table is then constructed with the peaks' retention times and assigned labeling. Then an unknown sample is run under identical conditions. By matching up retention times and presuming that no other compounds coelute at these expected retention times, the identity is made.

Unfortunately, the analyst may not always have a standard for the compound of interest. To help overcome this predicament, several protocols have been developed over the years to assist identification of peaks.

Coupling the chromatographic process to an information-rich spectroscopic detector, such as a mass spectrometer or Fourier transform infrared spectrometer, can provide nearly positive identification of unknown peaks. Unfortunately, every gas chromatograph cannot possess such detectors due to the added cost and complexity, and even these detectors rely on retention times to confirm peak identifications, as spectra for a handful of different closely-related compounds can appear to be quite similar.

Relative Retention Times

One identification process frequently employed in pesticide residue and gas analyses is to use "relative retention times" to help identify unknown peaks, or at least get a handle on its ID. A simple computation of ratios of retention times[1] for all of the peaks, relative to an established reference material[2], yields a number[3] that can be compared with published tables to help in the identification process.[4] These values are subject to a column temperature program profile, but are noteworthy for being independent of column dimensions and flow rate/headpressure.

Kovats and Linear Retention Index

Another process is labeled Kovats indices[5], and is most often applied to hydrocarbons. Here the n-alkanes are markers, and other hydrocarbons are assigned an index relative to where they elute relative to nearby n-alkanes.[6] For example, if benzene were to elute half way between n-Hexane (600) and n-Heptane (700), it would have a Kovats index of about 650. These indices are independent of column flow rate and column dimensions, but must be matched with the same column stationary phase. Originally, Kovats indices were limited to isothermal column conditions, but modifications[7] have been adopted for temperature programmed conditions, sometimes labeled as "linear retention index".[8] Compound identifications are made with either index by running a mix of straight chain hydrocarbons and computing the relative positions of the unknown peaks to the n-alkanes accordingly to the appropriate equation.

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[1] Varian chromatographic data systems since the CDS 111 and others have had the ability to perform this computation.

[2] Common reference peaks are Ethane for gases, Aldrin for pesticides and Octachloronaphthlene for PCBs.

[3] The calculation for "Relative Retention Time" (RRT) is :

$$RRT = \frac{\text{retention time}_{unknown} - \text{unretained peak time}}{\text{retention time}_{reference} - \text{unretained peak time}}$$

where "unretained peak time" is a correction for the time a component takes to migrate down the column without any interaction with the column stationary phase (usually air or methane). This adjustment compensates for different column lengths and flow rates.

[4] See for examples: Burke, J.A., Holswade, W., ***J. Assoc. Offic. Anal. Chem***, (1966) ***49***, 667, Erickson, M.D., ***Analytical Chemistry of PCBs***, (Butterworth Publishers, Boston, 1986), pp 190-192; Hammerstrand, K., ***Gas Chromatographic Analysis of Pesticides***, (Varian Associates, Palo Alto, CA, 1976), pp 54-55; Thompson, B., ***Fundamentals of Gas Analysis by Gas Chromatography***, (Varian Associates, Palo Alto, CA, 1977), page 16; and www.vici.com/hayesep/rettimes.php.

[5] Kovats, E., ***Helv. Chim. Acta***, 1958, ***41***, p. 1915.

[6] Kovats indices are computed by the formula:

$$KI = 100 \frac{(\log t_i - \log t_x)}{(\log t_{(x+1)} - \log t_x)} + 100x$$

where t is adjusted retention time (or retention time minus unretained peak time), and x is the number of carbons for the n-alkanes eluting just before the peak of interest.

[7] Van Den Dool, H., Kratz, P.D., ***J Chromatography***, (1963), ***11***, pp 463-471.

[8] Linear retention indices are computed by the formula:

$$LRI = 100 \frac{(t_i - t_x)}{(t_{(x+1)} - t_x)} + 100x$$

where t is adjusted retention time {or retention time minus unretained peak time}, and x is the number of carbons for the n-alkanes eluting just before the peak of interest.

Table 1. C_6-Benzene Hydrocarbon Portion of a typical Linear Retention Index

Component	Retention Time (min.)	Linear Retention Index
n-Hexane	51.183	**600**
t-3Hexene	51.280	602
c-3Hexene	51.329	603
t-2Hexene	51.426	605
2Methyl 2Pentene	51.523	607
3Methyl t-2Pentene	51.620	609
3Methyl Cyclopentene	51.669	610
c-2Hexene	51.766	612
3Methyl c-2Pentene	51.863	614
3,3Dimethyl 1Pentene	51.960	616
2,2Dimethyl Pentane	52.154	620
Methyl Cyclopentane	52.446	626
2,4 Dimethyl Pentane	52.688	631
2,2,3TriMethyl Butane	52.834	634
2,4Dimethyl 1Pentene	53.271	643
Benzene	53.611	650
n-Heptane	56.039	**700**

Lee Index

And more recently, Lee applied a similar scheme for polyaromatic hydrocarbons, with standard marker compounds of benzene (100), naphthalene (200), phenanthrene, (300), chrysene (400), and picene (500).[9]

All three of these approaches are very dependent on the choice of column stationary phase, but are relatively independent of column dimensions and column operating conditions, such as column temperature and flow rates. The scientific literature is full of tabulated indices for a variety of compound classes.

Retention Time Locking (RTL)

A major instrument manufacturer has been touting a process to perfectly match retention times between different columns of the same phase, and even different gas chromatographs, by adjustment of column inlet pressure to move a marker peak elution to a pre-established location.[10] The cited benefits include:

- reduction in method validation operations on a second instrument or different column with the same phase
- more direct comparisons with published data from literature, and
- use of databases to identify and "ensure greater confidence in results".[11]

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[9] Lee, M.L., Vassilaros, D.L., White, C.M., Novotny, M., ***Anal. Chem.***, (1979), ***51***(6), pp 768-773.

[10] US Patent Number 5,827,946 issued 1998. [http://patft1.uspto.gov/netacgi/nph-Parser?Sect1=PTO1&Sect2=HITOFF&d=PALL&p=1&u=%2Fnetahtml%2FPTO%2Fsrchnum.htm&r=1&f=G&l=50&s1=5827946.PN.&OS=PN/5827946&RS=PN/5827946]

[11] "Look in the Power of Gas Chromatography. It's Time", (Agilent Technologies, Palo Alto, CA, 2001). http://www.chem.agilent.com/scripts/pds.asp?lPage=178.

Two methods have been proposed. One involves an initial process of selecting a single analyte to be the locking compound, then making six chromatographic runs (including an initial blank) with five differing head pressures on the column to establish their retention time relationship. Then the operator manually enters these times and the target time value into a special software program that computes the new inlet pressure conditions for the column that are then downloaded into the gas chromatograph. This results in an adjustment for subsequent runs of the locking compound to the established time.

A second approach is to perform a series of runs with incrementing pressure until the target peak displays a retention time close enough to the target value – a potentially tedious process of repeat runs, especially with small increments in pressure over a wide range.

Either procedure intends to allow retention times of unknowns to be compared, without any further adjustment, with an established table of possible analytes. Then library hits are listed for possible peak identifications.

Precautions and Misguidance with RTL

The analyst must be extremely cautious in activating this process. Chromatographic conditions, including column dimensions, column temperature, and carrier gas must match those in the referenced search tables, as any deviation from the established column conditions are not corrected by this process. And the locking compound must be the largest peak within ± 20% of the target time. On top of these, the electronic pneumatics must be operated in constant pressure and **NOT** in constant flow or flow programmed modes.[12] Subtle variations in chromatographic conditions can impart significant changes in elution order of compounds and in relative retention of some analytes relative to the locking analyte, and generate potentially significant errors in the adjustment of the new head pressures.

Split/splitless capillary injectors rely on use of pressure control to maintain constant conditions at the head of the column, despite changes in split flow or split on/off.[13] Electronic pressure controllers can then compute column flow based on Hagen-Poiseuille Law[14], which relates column diameter, gas viscosity, pressure difference between the two ends of the column, and column length with flow. Errors in any of these variables will impact the accuracy of the computation. One variable has a major role in the end result - column diameter affects the flow rate by its **fourth** power**!** A subtle change in the diameter, say ± 4.8%, will introduce a ± 22% error in the computed flow rate. At the error extremes, the expected retention times can be as much as 56% off, under isothermal column conditions. This unpredictable error can render useless the computations needed to accurately readjust the headpressure needed to move a peak to a target time when switching between columns.

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[12] View www.chem.agilent.com/cag/servsup/usersoft/files/Autolock%20RTL.htm#. Then download autolock.exe. and view autolock.doc for system requirements and operations.

[13] Bramston-Cook, R., "Effectiveness of Electronic Pressure Control in Gas Chromatography", (Lotus Flower, Inc., Long Beach, 1995).

[14] Sutera, S.P., Skalak, R., "The History of Poiseuille's Law', ***Annual Review of Fluid Mechanics***, (1993), ***25***, pp 1-19.

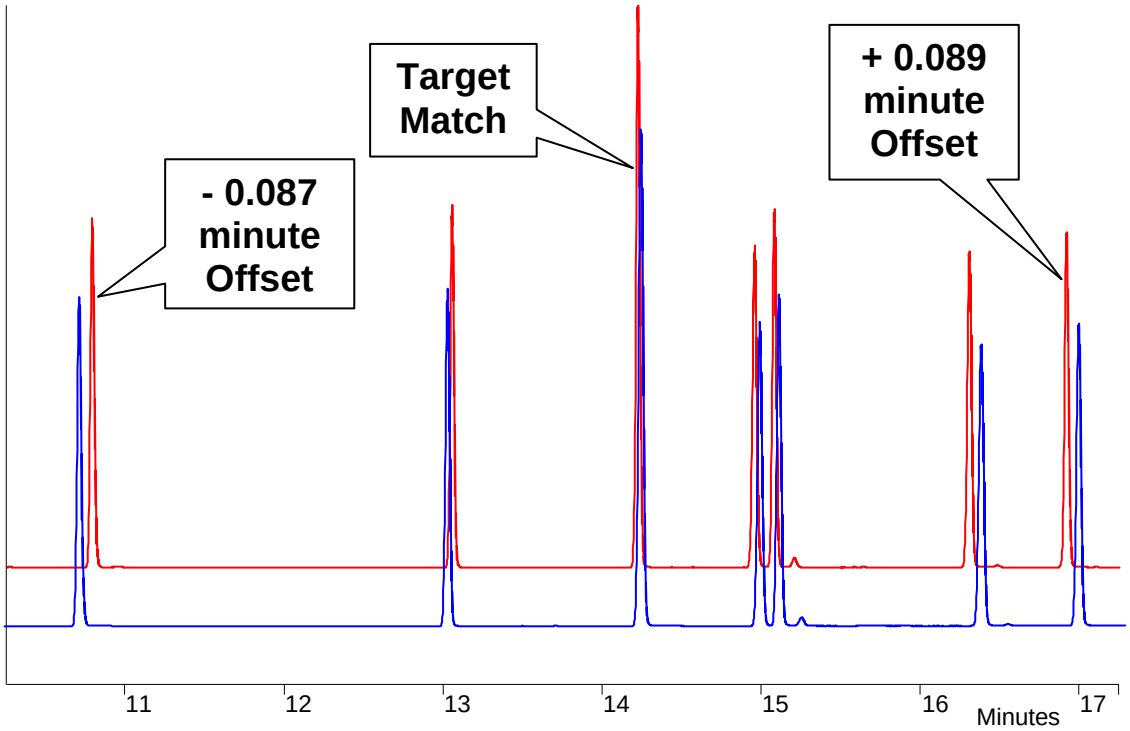

Figure 1. Chromatograms of C_5 - C_8 Hydrocarbons with temperature programming and two different column headpressures. Chromatograms are graphically adjusted to simulate a match for benzene retention times at 14.22 minutes between the two runs.

Column temperature program – initial -45 °C, hold for 1.50 min, ramp to 250 °C at 15 °C/min.
Red – Headpressure - 31 psiG, Blue – headpressure - 28 psiG.

Temperature programming is commonly used to shorten the analysis times of complex mixtures with a wide range of analytes. A higher temperature will cause the later-eluting analytes to move through the column a bit faster. This effect will often overwhelm the slowdown experienced during the run with a constant column headpressure mandated by retention time locking. When the headpressure is adjusted to bring a target peak into agreement between two different runs, this change will have varying impacts during column temperature programming with early peaks eluting too quickly and later ones too slowly. Only the target peak will have a perfect match.

Table II. Retention time locking is simulated by proportioning the shift in retention times with a differing headpressures so Benzene has a perfect match.

Analyte	31 psiG	28 psiG	Readjustment	Relative Shift
Ethene	3.542 min	3.936 min	3.708 min	-0.228 min
Ethane	3.726	4.139	3.901	-0.238
Propane	5.093	5.548	5.332	-0.216
2MePropene	7.376	7.887	7.722	-0.165
13Butadiene	7.490	8.004	7.841	-0.163
Butane	7.632	8.149	7.990	-0.159
Pentane	10.160	10.723	10.636	-0.087
Hexane	12.422	13.021	13.004	-0.017
Benzene	**13.591**	**14.228**	**14.228**	**0.000**
224TriMePentane	14.324	14.969	14.995	0.026
Heptane	14.448	15.089	15.125	0.036
Octane	15.674	16.351	16.409	0.058
mpXylene	16.285	16.959	17.048	0.089
oXylene	17.493	18.202	18.313	0.111

Chromatographic Experiments Not Viable with Retention Time Locking

Ethyne (acetylene), separated on an alumina PLOT column, is a notable example of dramatically shifting retention times related to the deactivation of the column from water, when most of the other hydrocarbons are not so impacted. This peak can readily jump from behind n-Butane on a new column, through this peak, and even show up in front of Methyl Propane, as moisture progressively degrades the column performance. Retention time locking offers no help in fixing Ethyne's wandering under these conditions.

Another chromatographic experiment that exhibits unusual retention shifts is with subtle changes in column temperature involving symmetrical molecules Carbon Tetrachloride and Neopentane, as well as the hydrocarbons Methylpropane (iso-Butane) and Methylbutane (iso-Pentane) on Molecular Sieve 5A columns. Thompson[15] reported that these compounds can dramatically alter their relative positions to Hydrogen, Oxygen and Nitrogen as a function of temperature. For example, Carbon Tetrachloride elutes well after Oxygen and nearly with Nitrogen at 110 ^{o}C, but emerges dramatically before Oxygen at 150 ^{o}C. Retention time correction by adjusting column headpressure for slight differences in column temperature could alter the peak assignments in this analysis, and induce serious mistakes when reporting results.

Column overloading can spawn unanticipated shifts in retention times, either sliding earlier – or later – when too much column loading occurs. Figure 2 demonstrates the magnitude of changes possible. Narrow-bore columns, especially with thin films, are very susceptible to this phenomenon. If retention time locking is set up with one sample loading, a different loading could shift the peak outside its expected retention window, and then the peak would be misidentified easily. And if this peak is the target, then all of the other peaks will be in error due to the differing load.

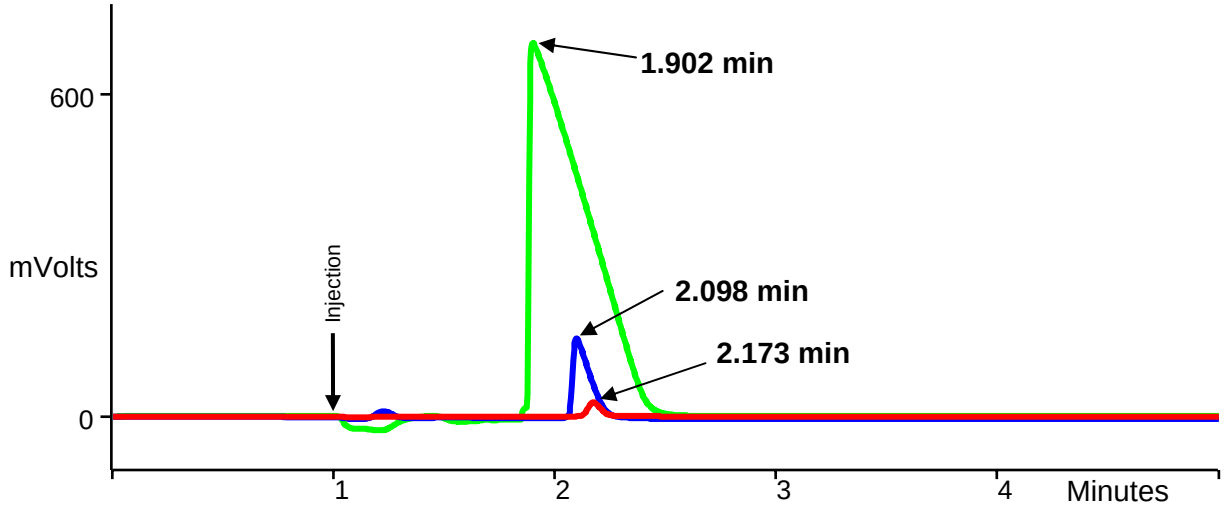

Figure 2. Column overload can severely impact peak shape and dramatically shift retention times as much as 0.27 minutes or more, as illustrated with measurement of pure Carbon Dioxide with argon carrier and various injection volumes. Red chromatogram is 0.01 ml injection, blue is 0.1 ml, and green is 2 ml loading. Column - Hayesep N, 60/80 mesh, 6' X 1/8", stainless steel, column temperature: 80 ^{o}C.

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[15] B. Thompson, *Fundamentals of Gas Analysis by Gas Chromatography*, (Varian Associates, Palo Alto, CA) 1977. pp 4-9.

This whole retention time locking process is based on fluid flow through a capillary column and cannot be applied to packed columns, including micro-packed. Nor can it work with many applications involving valving, especially with column switching, backflushing, heart-cutting and series/bypass configurations. Packed columns still have a very active and pertinent role in many chromatographic applications, especially in gas analysis. And valving techniques often involve heart-cutting and column switching with capillary columns. In either case, pressure adjustments to move retention times become extremely difficult to predict and then execute.

Identification of peaks in chromatography has always been a challenging detective game, and various schemes have been implemented to help in solving the riddle. Serious mistakes can be made if too much confidence is given to a single approach, with major degradations in the quality of the chromatographic result.

There can be no substitute for the conventional process of injecting standards and then unknowns in sequence with direct comparison of retention times, plus sensible use of compound-specific detectors to screen out interfering peaks, and careful employment of spectroscopic detectors to reconstruct the molecule. The final reliance must be made on operator judgment and experience for accurate assignment of the peak ID. Too many errors can be propagated by dependence on fudging experimental conditions to meet some arbitrary "standard" protocol, when so many unpredictable and uncontrollable variables can severely alter the end result.

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